

Response of Body Lice, Pediculus humanus humanus, L., to
Blackbody Radiation; with Notes on Their Antennal Morphology

By

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TABLE OF CONTENTS

	<u>Page</u>
ACKNOWLEDGMENTS	ii
LIST OF TABLES.	vi
LIST OF FIGURES	vii
LIST OF PLATES.	ix
ABSTRACT.	xiii
 CHAPTER	
I INTRODUCTION.	1
II REVIEW OF LITERATURE.	3
Reactions to blackbody IR radiation. .	3
Morphology of antenna sensors.	11
III METHODS AND MATERIALS	24
The test insect.	24
Behavior experiments	25
Straight arena experiments	28
Round arena experiments.	36
CO ₂ monitoring experiments	43
Reactions to an artificial finger and varied stimuli	52
Morphological studies of the antenna .	56
Light microscopy	56
Transmission electron microscopy . . .	57
Scanning electron microscopy	58

TABLE OF CONTENTS (CONTINUED)

CHAPTER		<u>Page</u>
IV	RESULTS AND DISCUSSIONS	59
	Straight arena experiments	59
	Round arena experiments	64
	CO ₂ monitoring experiments	67
	Reactions to an artificial finger and varied stimuli	82
	The significance of blackbody IR detection	85
	The antenna morphology	89
	Species comparison study	100
	Function of the antennal proprioceptive organs	102
	The tuft and pore organs	107
	The peg sensors	111
V	CONCLUSIONS	141
	REFERENCES CITED	145
	BIOGRAPHICAL SKETCH	152

LIST OF TABLES

<u>Table</u>		<u>Page</u>
1	Percent of the Total Number of Responding Lice Recorded at Each Compartment in the Straight Arena.	60
2	Percent of the Responding Lice Moving Toward the Blackbody and Aluminum Heaters in the Straight Arena	61
3	Average Number of Lice Responding When the Heaters Were On or Off in the Straight Arena.	62
4	Percent of Responding Lice Recorded in Front of Heated Areas at 33 C in the Round Arena	65
5	Percent of Lice Recorded as Responding to IR Stimulation in the Round Arena	68
6	Chamber Temperature and CO ₂ Concentration When BB or Al Heaters Were Maintained at 33 C for 65 min	71
7	Results of BB and Al Heaters Stimulation Upon 50, One-Day-Starved Body Lice.	77
8	Results on CO ₂ Output of Lice When Either the Al or BB Heaters Were Turned On and Off, Alternately.	79
9	Effect of Human Skin Air Emanations on CO ₂ Output of 50 One-Day-Starved Body Lice.	81

LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
1	Infrared Spectrum of Human Hand . . .	30
2	Infrared Spectra of Black Paint (Minnesota Mining and Manufacturing Co.) and Cellophane (Cigarette Package Wrapping)	32
3a, b	Infrared Spectra of Rubber Glove and Aluminum Foil, Heated to a 33 C Surface Temperature	34
4a, b, c	Round Arena Apparatus	39
5a, b, c	Infrared Spectra of Infrared Radia- tion Reflected at a 90° Angle from Aluminum and Black Paint.	41
6	A Schematic Diagram of the 2.5 cm Strip Arena	45
7	CO ₂ Chamber for Monitoring Lice Activity During Stimulation with Blackbody Infrared Radiation.	47
8a, b, c	CO ₂ Monitoring Flow Chart	50
9a, b	Flow Chart for Monitoring the CO ₂ Output of Lice During Stimulation with Human Skin Gaseous Emanations	54
10a, b, c	CO ₂ Output of Lice During Constant Stimulation Under Aluminum and Blackbody Radiators, or When Both Heaters Were Alternately turned On and Off with a 2.5 Min Off Period Inbetween.	73

LIST OF FIGURES (CONTINUED)

<u>Figure</u>		<u>Page</u>
11a, b	CO ₂ Output of Lice During Periodic Stimulation under Aluminum and Blackbody Radiators	76
12	Diagram of Adult Body Lice Antenna	90
13	Proprioceptive Sensors in the Antenna of Adult Body Lice.	103
14	Diagram Illustrating the Arrangement of the Blunt and Pointed Setae on the Tip of the Antenna of Adult Body Lice.	113

LIST OF PLATES

<u>Plate</u>		<u>Page</u>
1	Dorsolateral View of Left Antenna of Adult Body Louse.	92
2	Ventrolateral View of Left Antenna of Adult Body Louse.	92
3	Fourth and Fifth Segments of Adult Body Louse Antenna.	94
4	End View of Antenna Tip of 1st Instar Larva of the Body Louse	94
5	Antennal Tactile Hair, Innervating Dendrite and Neuron Cell; Axon Extends Toward Antennal Nerve	97
6	Campaniform Sensilla on the Second Segment of the Antenna of the Body Louse.	97
7	Campaniform Sensilla on the Second Segment of the Antenna of Body Louse, Under SEM . .	97
8	Campaniform Sensillae on the Second Antennal Segment, Demonstrating Their High Birefringency, Similar to the Hair Socket Between Them.	97
9	Same View as Plate 8, but Under Nomarski Differential Interference Contrast.	97
10	Internal Components of the Second Antennal Segment.	97
11	Double Chordotonal Organ on the Second Antennal Segment.	97

LIST OF PLATES (CONTINUED)

<u>Plate</u>		<u>Page</u>
12	Single Chordotonal Organ on the Second Antennal Segment.	97
13	Tuft Organ on Fifth Segment of Antenna of Adult Body Louse	110
14	Abnormal Tuft Organ on Fifth Antennal Segment	110
15	Pore Organ on Fifth Segment of Antenna. . .	110
16	Cross Section of Tuft Organ on the Fifth Segment	110
17	Cross Section of Tuft on the Fifth Segment	110
18	Cross Section Through Pore Organ on the Fifth Segment, OsO ₄ Fixation.	110
19	Antenna of 3rd Instar Larva of Body Louse Undergoing Molting.	110
20	Abnormal Louse with 2 Tufts Found on the Fourth Segment.	110
21	Distal Three Segments of the Antenna of 1st Instar Larva.	115
22	Tip of the Fifth Segment of Body Lice Antenna Showing How the Peg Sensillae Give the Impression of Thin-Walled Sensors	115
23	Tip of Antenna of Body Lice	115
24	Longitudinal Thick Section of Fifth Segment of Body Lice Antenna Showing Cell Bodies Underlying the Peg Organs . . .	115
25	Highly Magnified Pointed Seta from Adult Lice Antenna.	117

LIST OF PLATES (CONTINUED)

<u>Plate</u>		<u>Page</u>
26	Cross Section of a Pointed Seta from Adult Body Lice	117
27	Oblique Section of a Pointed Seta Through Basal Spot Area	117
28	Cross Section of Pointed Seta	117
29	Cross Section of Pointed Seta	117
30	Cross Section of Pointed Seta	117
31	Ultra-Thin Section of a Group of 3 Neuron Cells	123
32	Section Through Ciliary Region of Nerve Cells Innervating the Peg Sensilla.	123
33	Section of Dendrites Bundle Beyond the Ciliary Region.	123
34	Section of a 2-Dendrite Bundle of a Peg Sensor Cells.	123
35	Cross Section Through the Tip of Antenna of Body Louse	125
36	Same as Cross Section in Plate 35, but Distally to It.	125
37	Longitudinal and Somewhat Oblique Section Through the Base of a Blunt Seta.	129
38	Longitudinal Section of a Peg Sensor.	129
39	Section Through Bundle of 2 Dendrites Prior to Entering a Pointed Seta.	129
40	Cross Section Through Peg Sensors	131
41	Cross Section of Blunt Setae in the Proximity and Proximal to the Basal Spot.	131

LIST OF PLATES (CONTINUED)

<u>Plate</u>		<u>Page</u>
42	Cross Section of Blunt Setae in the Proximity and Proximal to the Basal Spot.	131
43	Cross Section of Same Sensor as in Plate 41, but Through the Basal Spot.	133
44	Cross Section of Same Sensor as in Plate 42, but Nearer to the Basal Spot.	133
45	Cross Section of Blunt Sensor, Distad to the Basal Spot.	135
46	SEM View of Nipple-Like Structure at the Tip of the Blunt Setae.	135
47	Longitudinal Section of the Nipple-Like Tip of a Blunt Seta	135
48	Different Aspects of OsO_4 Treated and Sonicated Antennae of Body Lice	138
49	Different Aspects of OsO_4 Treated and Sonicated Antennae of Body Lice	138
50	Different Aspects of OsO_4 Treated and Sonicated Antennae of Body Lice	138
51	Antenna of 3rd Instar Larva Molting into Adult.	138

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BLACKBODY RADIATION; WITH NOTES ON THEIR ANTENNAL MORPHOLOGY

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Detection of blackbody infrared radiation by the body louse, Pediculus humanus humanus, L., was demonstrated. Detection was studied using a good blackbody irradiator (black paint) and a poor one (aluminum foil), both having the same surface temperature. Detection and response were shown by the differential aggregation of lice near the blackbody irradiator. Change in lice activity due to infrared stimulation was quantified by using the CO₂ output of the lice as an index of activity. No response was obtained from stimulation by human skin gaseous emanations. Lice were shown to detect infrared independent of the convective heat arising from the host (blackbody irradiators).

The body louse antenna was described in detail using light microscopy and scanning and transmission electron microscopy. The components of the proprioceptive system of

the antenna were studied and their functions discussed. The tuft and pore organs were described and a survey of specimens from different laboratory colonies and from different parts of the world showed no variation in their sensor complement. The pointed and blunt setae on the tip of the antenna were found to lack permeable areas. Few and narrow pores were found on the walls of the pointed peg sensors, but none on the blunt ones. The presence of a basal spot, the absence of flask-shaped pores, the lack of dendrite branching and the absence of cuticular sheath on the peg prevent classifying these sensors as any described type of sensillae. These observations cast doubt on the claimed chemoreceptive function of these sensillae.

Evidence was presented to demonstrate that microtubule multiplication inside the dendrite takes place by the division, splitting and separation of the microtubules.

CHAPTER I

INTRODUCTION

The behavior of the body louse, Pediculus humanus humanus, L., has been studied extensively, especially its response to moisture, light, odors, surface texture and temperature. Response to blackbody infrared radiation has been studied extensively; however, it continues to be an area of research most controversial and confusing. The controversy has centered mainly on whether or not the louse reacts directly to the infrared radiation or to the convective warm air.

Previous research on louse response to blackbody radiation has measured locomotive patterns solely. Because of the inherent difficulty of quantifying results, using this method, studies used few individuals. Most studies have been of "tracks" of single lice. They have been mainly done under the influence and interaction of several environmental factors at a time, due to the difficulty of their isolation.

Behavioral studies of lice have been accompanied by

morphological descriptions of their sensory apparatus and attempts at designation of functions. Some of this work has been based on inferences made from the modification of behavior after antennectomy.

With the use of new techniques and light microscopes with higher resolving power, and of the newer electronic microscopes, transmission and scanning, a more detailed study of the antennal sensors can be performed. The study of sensors with these techniques has resulted in great advances. Studies of the ultrastructure of sensors usually provide sufficient information to permit assigning a function to a given sensor, with a high degree of confidence. However, new sensors are continually being found that do not fit any previous morphological-functional classification.

This investigation was undertaken to elucidate the problem of detection of blackbody infrared radiation by one-day-starved adult body lice. A second part of this research was a morphological study of the sensors on the louse antennae with emphasis on the so-called thin-walled sensillae on the fifth and terminal segment. These investigations were conducted mainly at the Insect Attractants, Behavior, and Basic Biology Research Laboratory, but also at the Insects Affecting Man and Animals Research Laboratory, ARS, USDA, at Gainesville, Florida.

CHAPTER II

REVIEW OF LITERATURE

Reactions to blackbody IR radiation

Insect reactions to temperature are a well-documented subject (Bursell, 1964). This environmental factor has been usually presented to the insects as either floor or air temperature. The resultant response has generally been due to the interaction between temperature and moisture; however, there are responses to temperature as such which have been demonstrated for several species (Bursell, 1964).

Reactions of blood-sucking insects to the host "warmth" have also occupied the attention of innumerable investigators. A host animal, such as a homoiotherm, dissipates heat in the following ways: water evaporation (respiration, sweat), conductive and convective heat, and as blackbody infrared radiation (hereafter abbreviated blackbody IR radiation). It is not illogical to conjecture that blood-sucking insects use one of these forms of heat loss as a token stimulus for host detection and, ultimately, orientation. It has been demonstrated that several species of

snakes use blackbody IR radiation for host detection (Noble and Schmidt, 1937), and that the eye of slugs (Mollusca, Gastropoda) responds to blackbody IR radiation (Newell and Newell, 1968). It was also demonstrated in several instances that insects detect other forms of IR radiation, i.e., IR from other sources. Callahan (1965, 1967) has proposed that certain insect sensillae act as dielectric antenna and therefore are tuned to discrete IR frequencies given off by molecules. Bruce's (1969) research with the spiny rat mite, Laelaps echidnida, Berlese, supports this theory. Recent research by Levengood and Eldumiati (1971) on moth attraction by far IR lasers also supports these theories. Evans (1964, 1966) showed that buprestid beetles of the genus Melanophila can detect forest fires several kilometers away by the use of the IR radiation produced by these fires.

To demonstrate that insects do use the blackbody radiation from their warm-blooded hosts as a stimulus has been most difficult, confusing and, in some instances, frustrating to entomologists. The problem encountered by any investigator studying IR as a stimulus for host detection has been of the same nature as the one met by physicists working on this region of the electromagnetic spectrum, that is, the low energy of the radiations (Callahan, 1967). The large number of failures, misinterpretations and

contradictory results obtained from studies on this area are due to the lack of understanding of the physics of this region and/or lack of proper or efficient equipment for defining properties or conditions encountered when trying to measure or reproduce IR radiation. Quite often the problem stems from failure to separate the basic components of heat, i.e., convection, conduction and radiation. Consequently, many authors talk about the host's "warmth," without separating these components.

Mosquitoes' response to the host's "warmth" has been a popular subject among mosquito specialists. Parker (1948), working with Aedes aegypti, L., concluded that this mosquito is not attracted by the host's "warmth." Peterson and Brown (1951) carried out a series of experiments with the same mosquito, using billiard balls at different temperatures. From these experiments they concluded that the balls' attractiveness increased as they were heated to 110 F, but a 130 F ball was less attractive than one at 110 F. I believe this implies good temperature discrimination. When the mosquitoes were separated from the balls by both a KRS-5 filter (transparent in the 25-40 μ m region) and at a distance of about 10 cm there was no attraction. These authors concluded that convective heat was the factor which made the warm objects attractive to mosquitoes. However,

Magnum and Callahan (1968) showed that this same mosquito was attracted to a source of IR radiation, or even to the radiation reflected from an aluminum sheet (known as a good IR reflector).

Bedbug (Cimex lectularius, L.) behavior, as studied by Rivnay (1932), demonstrated that "heat is an important factor in stimulating bed bugs to obtain food." This author also demonstrated that the bugs could detect the heat from a hand at a distance of 4 cm, and that they were repelled when the temperature was too high (43 C). Aboul-Nasr and Erakey (1967) working with the same bug concluded similarly, and believed that convective heat was the important factor. Also, they concluded that the orientation of this insect to heat was mainly achieved by klinotaxis and klinokinesis. Fraenkel and Gunn (1961) defined klinokinesis as: "Frequency or amount of turning per unit time dependent on intensity of stimulation"; and klinotaxis as: "Attainment of orientation indirect, by interruption of regularly alternating lateral deviations of part or whole of body, by comparison of intensities of stimulation which are successive in time."

Wigglesworth and Gillett (1934) found that Rhodnius prolixus, Stål was attracted to its host by the warmth diffusing from it. Blinded specimens could go straight to a

test tube with warm water 3-4 cm away, but antennaless specimens didn't show this reaction. They believed that the thermal sensors were located chiefly in the antenna, but could not locate them.

Reports on behavior of anoplurans to warmth included a number of scattered observations and experiments, and a few extensive studies. Frickhinger (1916) (as quoted by Rivnay, 1932) stated that the body louse had a sense of heat (wärmesinn). Howlett (1917) made some interesting observations on head and body lice and their temperature reactions. He described the change in locomotive activity, both quantitatively and qualitatively, of lice stimulated by the radiant heat from a tube at 35 C. Crab lice, Phthirus pubis, L., as tested with the warm tube (also called a "finger"), presented a reaction similar to body lice. Martini (1918) reported that body lice aggregated under a source of radiant heat, even if it meant their moving from the warmer to the cooler part of a temperature gradient (quoted in Fraenkel and Gunn, 1961).

The next important work in this area was written by Homp in 1938. This study illuminated many of the behavioral patterns of lice, but at the same time produced a great number of contradictory conclusions. She studied the temperature changes of the arena floor, the air and the lice

when a warm "finger" was brought close (in some cases she worked with temperatures not usually encountered by the lice, such as 49 C). It was known that lice would follow a warm "finger." Homp blinded several lice and they still would follow the "finger," but when it was quickly withdrawn, the lice would abandon their orientation toward the "finger." Homp concluded that lice did not respond to radiant heat. This author concluded that the movement of lice towards a warm "finger" was consistent with klinotaxis.

The Wigglesworth (1941) paper on the sensory physiology of body lice is a classic on the study of sensory physiology by means other than electrophysiology. In this work he studied reactions of lice to temperature, humidity, smell, contact and light, and included a morphological study of the lice sensors. He concluded that the mechanism of orientation to temperature consisted of an increase of random turning movements, in other words, klinokinesis. He stated that "there is no evidence that the louse is 'attracted' by a favourable stimulus, although it may show a directed orientation where there is a steep gradient of stimuli."

In order to test the ability of lice to detect IR radiation he performed the following experiment:

a circular tin, 9 cm in diameter, was lined with aluminum foil, and on one half of the wall this was covered with thin cellophane gummed to

the surface. The thin cellophane covering makes little difference to the conduction of heat and consequently to the gradient of air temperature from the walls to the centre of the arena. But it makes a great difference to the radiant heat. The emissivity of the aluminum covered by cellophane is almost equal to that of a dull black surface; the emissivity of the aluminum alone is only about 5% of this.

The walls of the arena were heated equally or differently. Using this arrangement, he released lice on the center of the arena. He measured also the temperature of the air adjacent to the walls. All of these experiments were conducted under good light conditions. He recorded the "tracks" of lice leaving the cluster at the center of the arena and moving toward the walls. He did not find any difference between the lice orientation toward the cellophane (good irradiator) and the aluminum (poor irradiator) surfaces. Unfortunately, he did not record the path of the lice after they had reached the walls. A louse walks in the same orientation which it had when dropped on the arena floor. It is not until the louse is close to a warm body that it moves directionally (Homp, 1938; Weber, 1929).

Wigglesworth may have been able to obtain more information from these observations. His final conclusion was that "the response is always to air temperature, there is no response to radiant heat from objects at 20-45 C." Homp (1938) and Wigglesworth (1941) observed that lice deprived of their

antennae would come very close to a hot object, while normal lice would avoid objects at 40 C; that is, the antenna possesses sensors that are sensitive to high temperatures. Wigglesworth (1941) showed that antennaless lice could discriminate different temperatures only when this difference was great, i.e., between 20 and 30 C.

The response of blood-sucking insects to heat has been measured with different techniques. One of the most common has been by observing some type of behavior associated with feeding. Hopkins (1964) used the "thrusting movement of the extended proboscis" of the stable fly (Stomoxys calcitrans, L.); similar responses were used by Rivnay (1932) with the bedbug and Wigglesworth and Gillett (1934) with Rhodnius. Other forms of behavior associated with feeding have been used, such as questing of sheep ticks, Ixodes ricinus, L. (Lees, 1948) and spiny rat mites, Laelaps echidnida (Bruce, 1969). Aboul-Nasr and Erakey (1967) used the awakening from akinesis of bedbugs as a positive response to heat.

Another form of monitoring response behavior to heat has been recording the number of insects reaching the stimulus area, such as the work with mosquitoes (Parker, 1948; Peterson and Brown, 1951; Magnum and Callahan, 1963). Berry and Kunze (1970) quantified the response of stable

flies to blackbody IR radiation by means of their flight activity, an indirect method. Turner and Charity (1971) determined activities of moths (a non-blood-sucking insect) by continuously monitoring their carbon dioxide output.

A widely used method has been recording the "tracks" of the insects and analyzing them. This method has the advantage of providing insight into the type of orientation, but at the same time it suffers from many inherent problems. It is difficult to quantify and has to be conducted with one or a few individuals at a time. In addition, it has to be performed under well-lighted conditions and this is an unnatural condition to many blood-sucking insects. Also, the investigator has to be in close proximity to the experiment to record the response, and it is difficult to exclude his own radiation and other dissipating factors (i.e., odor, CO₂, breath) from influencing the test insect.

Morphology of antenna sensors

The varied array of sensors on insect antennae is a source of speculation as regards their functions and has been a challenge to study since the early days of experimental biology. Experiments to test the hypothesis that the antennal sensors are involved in olfaction were conducted as early as 1734 (Dethier, 1954). Electrophysiological

techniques have proven the hypothesis that olfactory sensors are located principally on the antenna. These techniques have also shown that there are other types of antennal sensors with functions other than olfaction, i.e., temperature, sound, mechanoreception.

Knowledge of the ultrastructure of insect sensillae has increased greatly in the last decade. These rapid advances can be linked to the popularization of the electron microscope and improvement of sample preparation techniques. Fixation and sectioning of sensory structures on insect antennae present special problems correlated with the hard cuticle and small diameter (Slifer, 1968). The morphology of the body louse antennae was studied first by Keilin and Nuttall in 1930. These authors reported that the main structures on the antenna of the first stage larva "examined in vivo" showed upon clearing the following structures: "two bicolopal and one monoscolopal chordotonal organ" (on the second segment), "muscles on the basal segment, antennal nerve divided into two branches, . . . sensory papillae with their ganglia" (on the tip of the fifth segment), and "sensory tuft of 4 hairs with tube penetrating into sensory ganglion" (on the fourth segment).

Wigglesworth (1941) described in greater details the antenna of the body louse, but in this case it was of the

adult louse. According to this author, the body louse antenna consists of five segments bearing three types of sensillae:

Peg organs. There are nine or ten on each antenna: three sharply pointed, lying dorso-lateral, and six, or usually seven, of varied length, with rounded tips, lying medial and ventral. In section they are seen to be exceedingly thin-walled. Below each is an elongated group of about six sense cells, the distal processes of which unite to form a filament that can be traced into the cavity of the peg.

Tuft organs. In the adult louse there are three tuft organs on the dorso-lateral aspect of the fifth segment and one at the tip of the fourth segment or its outer side. Each consists of a minute cone arising from the floor of a saucer-shaped depression. At the apex of the cone there is a tuft of four tiny delicate hairs which stain weakly with haematoxylin. These hairs appear to arise from a delicate membrane. Below this is a little oval cavity through which runs a deeply staining rod or filament attached at the point where the four hairs unite. A curved tubular thread connects this rod with a group of five or six sense cells.

Tactile hairs. These are of the usual type and consist of a slender bristle arising from a socket below which are trichogen and tormogen cells and a single sense cell with axon fibre. They vary somewhat in number, but there are usually 5-7 on segment 1, 8-10 on segment 2, 5-7 on segment 3, 3-4 on segment 4, 3-4 on segment 5.

Scolopidial organs. In segment 2 there are Johnston's organ and some chordotonal organs, which will not be described in detail.

The two previous descriptions disagree on the number of tuft organs on the antenna. This difference is the result of the studying of immature and adult forms of the same species. Miller (1969) reported a discrepancy between his study and that of Wigglesworth's on the number of tuft

organs on the adult body louse antenna. According to Miller, there was only one organ on the fifth segment and another on the fourth. However, he found two pore organs in the locations where Wigglesworth had reported the tufts. Miller extended this study to several races of body lice from North America and has obtained the same results (personal communication, 1970).

The morphology of the tuft organs on the antenna of the body louse have not been studied in detail. Very few other tuft organs have been studied. Hafez (1950) described two types of sense organs from the mesothorax of the house fly (Musca domestica, L.) larva, and he believed they were hygrometers. One type is in the form of a "minute brown circular structure about 10 μ in diameter"; it possesses a thin dome-like cuticular covering. The second type consists of "three minute hairs arising from the floor of a slight depression of the cuticle. Below these hairs is a small oval cavity through which extends a somewhat deeply staining rod." These hairs were about 6 μ m in length (Hafez, 1950). Roth and Willis (1951) described some tufted basiconic sensillae, bearing a varied number of prongs 5-15 μ m in length, from the antennae of several species of Tribolium. They concluded these sensors were hygrometers.

It is interesting to notice the similarity between

these two tufted-sensors and the ones on the body louse. The major difference is their dimensions; in Pediculus, these hairs are approximately 2-3 μm in length (Miller, 1969). Wigglesworth (1941) stated that the tuft organs on the body louse are hygrometers: the same function as in the species of Tribolium (Roth and Willis, 1951). However, Miller has recently indicated (personal communication, 1970) that he has evidence that these tuft organs are not moisture receptors of the louse, as reported by Wigglesworth (1941).

Wigglesworth (1941) coated the peg organs on the apex of the fifth segment and concluded that these "exceedingly thin-walled sensors" were involved in olfaction. These pegs have often been cited in the literature as excellent examples of thin-walled sensillae (Slifer, 1968; Schneider, 1964; Dethier, 1954). Schneider (1964) made reference to the Wigglesworth experiment as one of the few cases in which "it was possible to identify olfactory sensilla basiconica on antenna" with a sealing method. However, Chapman (1969) criticized this methodology by stating that "the identification of olfactory receptors is often uncertain because it is based only on the results of ablation experiments."

Classification of antennal sensors, as to sensory mode, has been based mainly on their morphology. A function can

be assigned to a sensor, based on its morphology, especially its ultrastructure. Such classification has often been demonstrated to be correct by electrophysiological studies.

The peg organs at the tip of the body louse antennae have been classified as basiconic sensillae. Schneider (1964) described basiconic sensillae as follows: "omni-present trichoid sensilla without any specialized basal membrane. If present together with sensilla trichodea on an antenna, they are relatively shorter and usually have a thinner wall. From one to several nerve fibres have been observed in connection with these organs." Sinoir (1969) described them as being generally small or very short bristles of conic shape, with no articulation at the base or mechanoreceptive structures, and possessing several sense cells under the peg.

Previous to the study of basiconic sensillae with the transmission electron microscope, it was discovered that these pegs stained with certain dyes. The dyes penetrated the pegs throughout the surface of some of them, but, in others, only through the distal tip (Slifer, 1954a). Slifer, Prestage and Beams (1957) confirmed this earlier observation with the use of the transmission electron microscope. These authors found that those basiconic sensillae staining through the tip had an opening (hole) at the

tip through which the distal processes of the sensory cells were exposed. Later, these same authors reported that the surface of the short, thin-walled basiconic pegs on the antenna of grasshoppers was perforated by a large number of pores between 0.1 and 0.2 μm in diameter, through which the distal tips of the dendrites were exposed. These were the sensillae which stained throughout their surface (Slifer, Prestage and Beams, 1959). These two types of basiconic sensillae are referred to as thick-walled (with an opening at the tip) and thin-walled (with pores throughout its surface). The classification is not based only on the thickness of the cuticle, but on other characteristics as well (Slifer, 1970).

A basiconic sensillum, whether thick- or thin-walled, is innervated by one or more bipolar nerve cells which send dendrites into the peg and axons to join the antennal nerve (Slifer, 1970). When the dendrite leaves the cell body, it assumes certain peculiar characteristics. Near the nucleus, it narrows down and takes the form of a cilium with nine pairs of peripheral double fibrils (doublets), but lacking a central pair. A basal body with a periodic structure arises from the cilium toward the nucleus. The cross section of this basal body indicates that it is composed of nine triple fibrils (triplets). When they enter the ciliary

region they become the doublets. The doublets of the ciliary region separate and enter the dendrite, which travels towards the peg; here the fibrils become indistinguishable microtubules (Slifer, 1970; Slifer and Sekhon, 1969).

Slifer and Sekhon (1969) presented evidence that the microtubules were a continuation of the fibrils; they usually split and so their number increased above 18. Microtubules in the dendrites have been usually found in numbers close to 18, or in multiples such as 36, 72, etc. The variation in number may be a result of the microtubules not all dividing or doing so at different levels (Slifer, 1970).

The trichogen cell which surrounds the sense cells usually forms vacuoles around the basal and ciliary regions of the dendrite. The tormogen cell which lies closer to the cuticle under the peg also forms some vacuoles around the dendrite (Moeck, 1968). The dendrites are surrounded by a cuticular sheath from the point where the fibrils become microtubules (Slifer, 1970). From this point toward the peg is where the major difference exists between the thick- and thin-walled sensillae, i.e., the ultrastructure of the peg.

There are several excellent review papers on the ultrastructure of thin-walled sensillae for they have been studied more than any other sensor on the antenna. Therefore, there is no need to discuss them in detail. The

following description of these sensors was synthesized from these review papers (Slifer, 1968, 1970; Sinoir, 1969; Schneider, 1969).

The surface of the thin-walled sensillum is perforated by a great number of pores which open to a spherical chamber; from this chamber tubules penetrate the cuticle toward the hair lumen and are in close proximity to the dendrites. This type of sensillum may be innervated by one or as many as 60 dendrites. There is a cuticular sheath surrounding the dendrites and it is invaginated from a spot at the base of the peg (basal spot). During molting, the sheath is shed through this hole. The dendrites cross the cuticular sheath near its distal end and enter the lumen of the peg. Branching may occur near the base or at different levels of the peg; and may produce small dendrites with many microtubules or as few as one.

Crystal violet, methylene blue and silver compounds have been the most commonly used chemicals to detect the presence of holes on thin-walled sensors with the use of the light microscope. These compounds also penetrate the basal spot on the peg. The pores have been studied with the transmission electron microscope in several species. Thin-walled sensillae have been demonstrated by electrophysiological techniques to be the main olfactory organs in insects.

Thick-walled basiconic sensillae possess a lumen filled with few dendrites, usually close to five. In some cases, it has a double lumen, one portion being occupied by the dendrites (Slifer, 1970; Foelix, 1970). The presence of a lumen throughout the bristle is the main characteristic that distinguishes a thick-walled basiconic from a mechanoreceptive hair (Sinoir, 1969). A thick-walled sensillum has a single opening at its tip, through which the distal ends of the dendrites are exposed and these dendrites may even extend slightly beyond the tip (Slifer, 1970). Slifer (1967) found that thick-walled sensillae in earwigs (Forficula auricularia, L.) had a broad pad-like tip which stained deeply with crystal violet.

A tubular sheath of cuticle is invaginated from the open tip of the peg which passes down the peg lumen. This cuticular sheath narrows at the base of the peg, and then widens below the base (Slifer, 1961). Slifer, Prestage and Beams (1957) showed that during molting the cuticular sheath is pulled out through the open tip and shed in this fashion. The occurrence of an open tip has been demonstrated by several techniques such as dye penetration using crystal violet (Slifer, 1960), methylene blue (Slifer et al., 1959; Foelix, 1970) and different silver preparations, such as silver nitrate (Slifer et al., 1957), the scanning electron

microscope (Foelix, 1970), and the transmission electron microscope (Adams et al., 1965; Stürckow et al., 1967).

Slifer, Prestage and Beams (1957) showed that when a fresh antenna was examined on a slide with olive oil and pressure applied to the cover slip, a drop of fluid would be forced out through the tip and if the pressure was held long enough, there was a crumpled membrane seen when the pressure was released. The majority of the thick-walled sensillae described exhibit longitudinal fluting (Moeck, 1968), longitudinal surface striae (P. S. Callahan, personal communication, 1970) or oblique striation spiraling around the shaft of the hair (Foelix, 1970).

Thick-walled sensillae had been found in many species of insects and other arthropods (Foelix, 1970). They were reported from different parts of the body besides the antenna (Slifer, 1970). Slifer (1954b, 1970) demonstrated that the thick-walled pegs on the grasshopper are the "receptors for the common chemical sense and are stimulated by strong repellent odors." Thick-walled sensillae have been demonstrated to be contact chemoreceptors in several flies, such as Phormia regina, Meigen (Dethier, 1955), Stomoxys calcitrans (Hopkins, 1964). Foelix (1970) stated that "an open tip is a strong argument for a chemoreceptive function."

Moeck (1968) described a hair sensillum from the ambrosia beetle (Trypodendron lineatum, Oliver) as being thick-walled, having no open tip, but having instead a few flask-shaped holes over the surface. These holes had a diameter of 200 Å; two dendrites entered the hair lumen and branched in its distal half.

Thurm (1964) described in detail the ultrastructure of mechanoreceptive sensillae of the honey bee, Apis mellifera, L. The articulated hairs are innervated by mechanoreceptive dendrites which are attached to a tubular electron-dense body at the base of the hair. Adams, Holbert and Forgash (1965) found that one of the dendrites innervating the thick-walled tarsal and labellar hairs of Stomoxys calcitrans was attached to the hair and this could be a mechanoreceptor. It is known that similar hairs in Phormia regina respond to mechanical as well as to chemical stimuli (Grabowski and Dethier, 1954). Foelix and Axtell (1971) reported that certain setae on the tarsi of the tick Amblyomma americanum (L.) are innervated by both mechanoreceptive dendrites ending as a tubular body at the bristle hair, and dendrites occupying the hair lumen.

A critical review of the literature on sensor classification demonstrates the inconsistency among different authors. For instance, Schneider (1969) considered sensillae

trichodea as being "long, thick-walled hairs or pegs." Lewis (1971) described the trichoid sensillae on the antennae of Stomoxys calcitrans as being "the largest and most conspicuous of the antennal sensillae, curved structures tapering to a point." They had about 500 pores on their walls and a cuticle 0.5 to 1.0 μm thick. It appears that recent workers consider Slifer's thick-walled sensillae to be trichodea. Sensillae nomenclature is in such a state of instability and so many different types and variations are constantly being described, that many authors avoid using any established sensillae names, and prefer using setal maps (Foelix and Axtell, 1971) or give them names associating their gross morphology with common objects (Callahan, 1969, and personal communication, 1970).

CHAPTER III

METHODS AND MATERIALS

The test insect

Body lice were obtained from the rearing facilities at the Insects Affecting Man and Animals Research Laboratory of the United States Department of Agriculture, Gainesville, Florida. Rearing procedures have been published elsewhere (Smith and Eddy, 1954). The lice were held on 2-inch square patches of black corduroy. Black cloth was used because the eggs and lice are more visible on the dark background. The patches with lice were kept in stainless steel dishes and maintained in cabinets at a relative humidity of 60 percent or less and at about 25 C. They were fed on rabbits twice each day, morning and afternoon. The patches were placed on the shaved ventral side of the rabbits. After 30 minutes the patches were rubbed against the rabbit skin and the lice were easily picked up as they clung to the cloth.

Lice from the standard colony were used for the behavioral experiments and for morphological studies, unless otherwise stated. The standard colony was started in 1942

from specimens collected from humans in Washington, D. C., and has been maintained in the laboratory since that time. Lice from three other experimental colonies were used for morphological comparisons. These were: Korea, collected in 1951 and selected for DDT resistance; Freetown, collected in Sierra Leone in 1956 and selected for Lindane resistance; and Burundi, collected in 1970 and selected for Malathion resistance (M. M. Cole, personal communication, 1970)

Behavior experiments

For the behavior experiments, only adult lice were used, 3 to 5 days after their last molt. Since sexing was difficult, both females and males were used. Fully engorged lice were obtained and then starved for 24 hours (\pm 12 hrs.) before using them in any experiment. No apparent physiological harm from this procedure was observed. Lice can survive without food for several days (Buxton, 1946). All the tests were conducted in rooms at 22 C and 50 percent relative humidity.

The behavior experiments dealt with the response and detection of blackbody IR radiation by body lice; hence, attempts were made in all tests to control carefully other stimuli. Lighting effects presented many problems. Wigglesworth (1941) showed that lice moved toward dark places or

dark objects, and that slight differences in the light received from different directions elicited greater responses if the lice were exposed to a general low intensity illumination. Therefore, the experiments were conducted in a closed room with the lights off, except when light was necessary. Preliminary control experiments demonstrated that the lice were reacting to the extremely low intensity light coming under and around the door cracks; therefore it was necessary to tape black cloth around these areas to completely eliminate any stray light. Obviously, running the experiments in the dark presented many problems and restricted gathering certain information; however, darkness is a natural condition for the lice. Buxton (1946) criticized Wigglesworth's work (1941) on lice orientation and reaction to different stimuli for running them under good illumination, because this was "an unnatural condition to them" (to the lice).

Wigglesworth (1941) demonstrated that lice reacted to several odors, including sweat, other lice or their excreta. In order to eliminate any interaction of odor with the response to blackbody IR radiation I had to artificially reproduce the skin blackbody IR radiation. Human skin is an ideal blackbody, since its emissivity approaches unity from about 3 μm to 15 μm (Hardy, 1954, as quoted by Barnes,

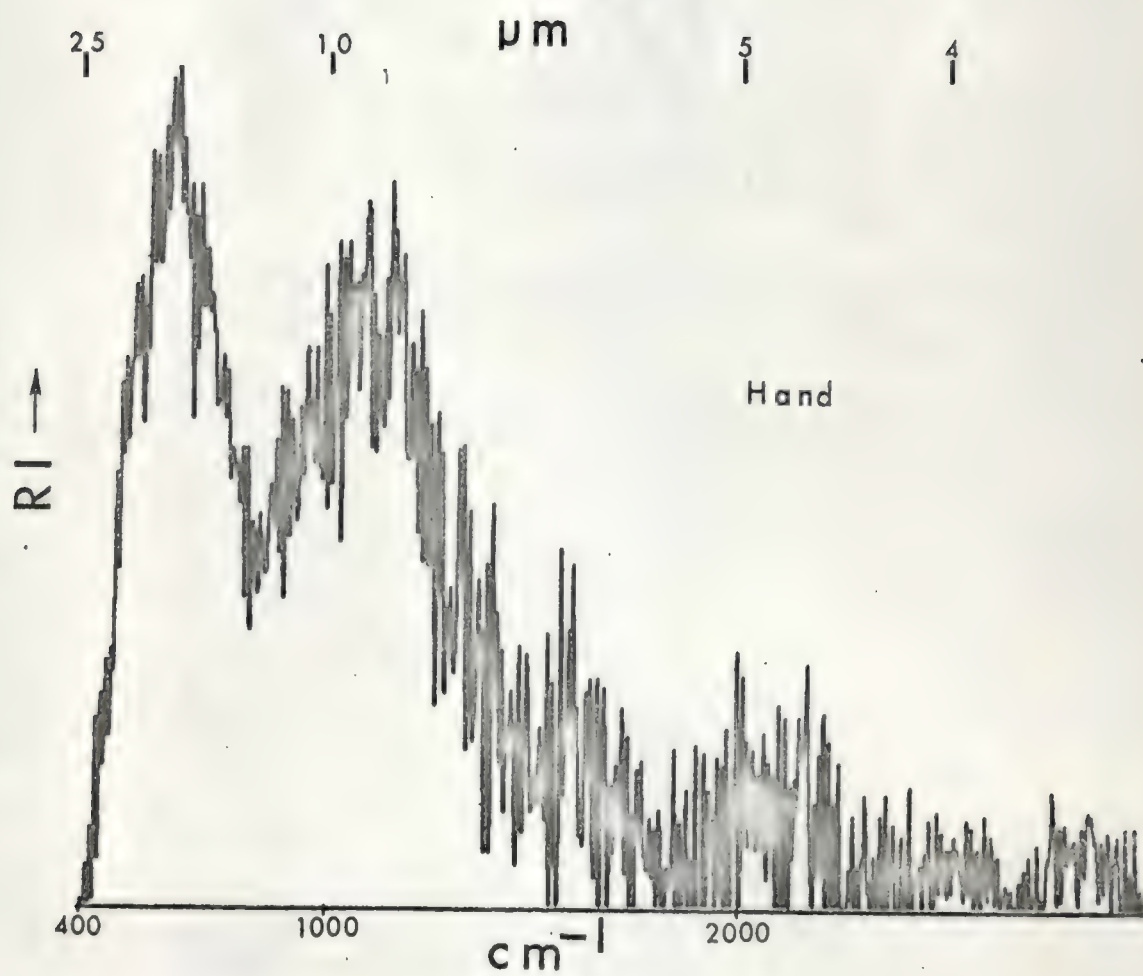
1967). Mitchell, Wyndham and Hodgson (1967) quoted an emissivity mean value of 0.997 ± 0.001 for the skins sampled. They also confirmed Hardy's statement that "the emissivity of skin in its thermal emission band does not depend on the skin pigment" (Hardy, 1934, as quoted by Mitchell et al., 1967). The blackbody spectra of several materials at 33 C and human skin were determined using an FTS-14 interferometer (Digilab-Block Engineering, Inc.). This is an infrared Fourier transform spectrometer which utilizes a Michelson interferometer. Materials to be analyzed were attached to a 1-inch square, 5-watt tape heater (Electrofilm, Inc.) covered with aluminum foil. The materials were heated to 33 C (surface temperature) by means of a temperature controller (Alton Electronics Co.) which could control the temperature within 0.25 C of the set temperature. Surface temperature readings were made with a thermistor digital thermometer model 501 with a scale of hundredths of degree C (± 0.15 C accuracy, 0.05 C repeatability; United Systems Corp.). Some of the materials tested were: Aluminum foil, Plexiglass^(R), Saran wrap^(R), cellophane (cigarette package wrapping), black electrical tape, 3M black paint (Velvet coating No. 101-C10, emissivity = 0.97; Minnesota Mining and Manufacturing Co.), and rubber glove (Surgeon's glove, grade A; Perry Rubber Co.). The blackbody

spectra of human skin, 3M black paint, rubber glove, aluminum foil and cellophane are shown in Figures 1, 2a, 2b, 3a and 3b. The 3M black paint at 33 C approximated best the human skin and it was selected to serve as "human skin" in further tests. A temperature of 33 C was chosen as a standard human skin temperature. Mitchell et al. (1967) stated that the skin temperature varies considerably, but it is known to be in the region of 25-40 C; Barnes (1967) showed that the average palm surface temperature was 32.4 C (range 29.7-34.3 C).

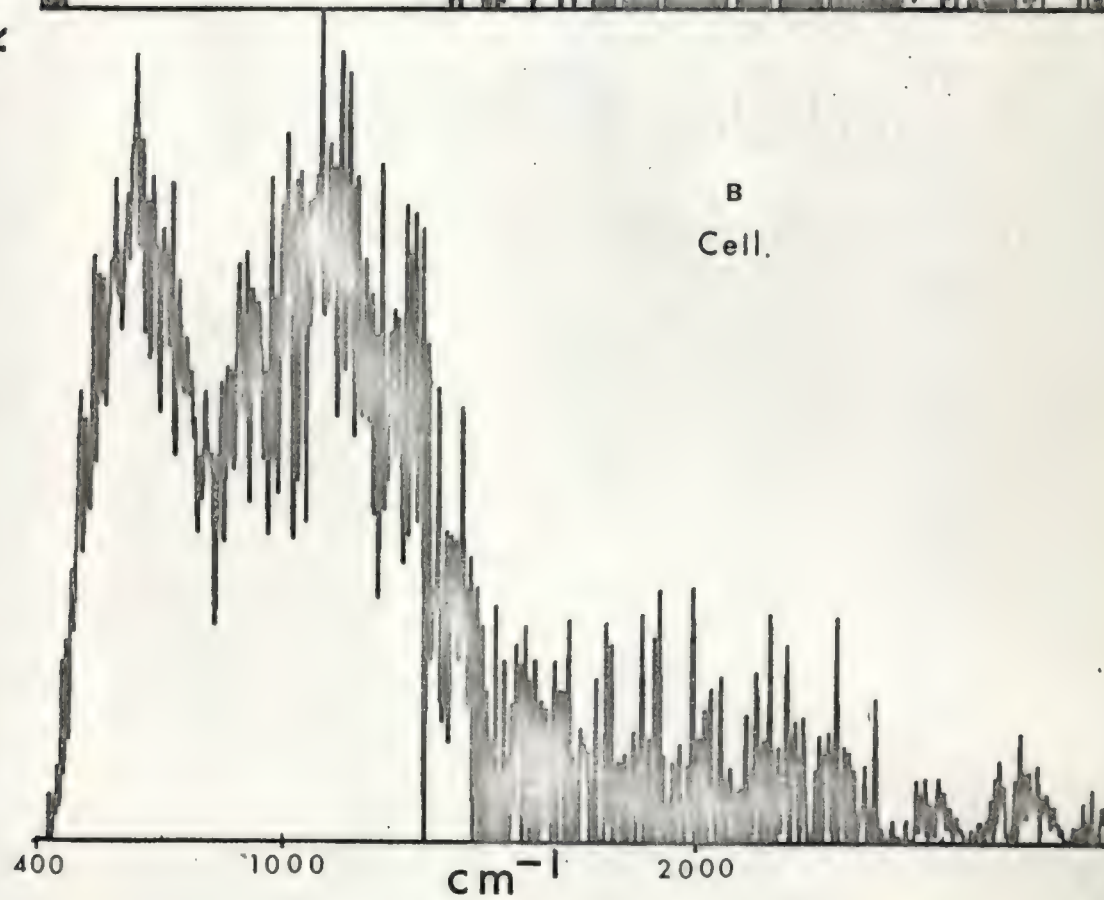
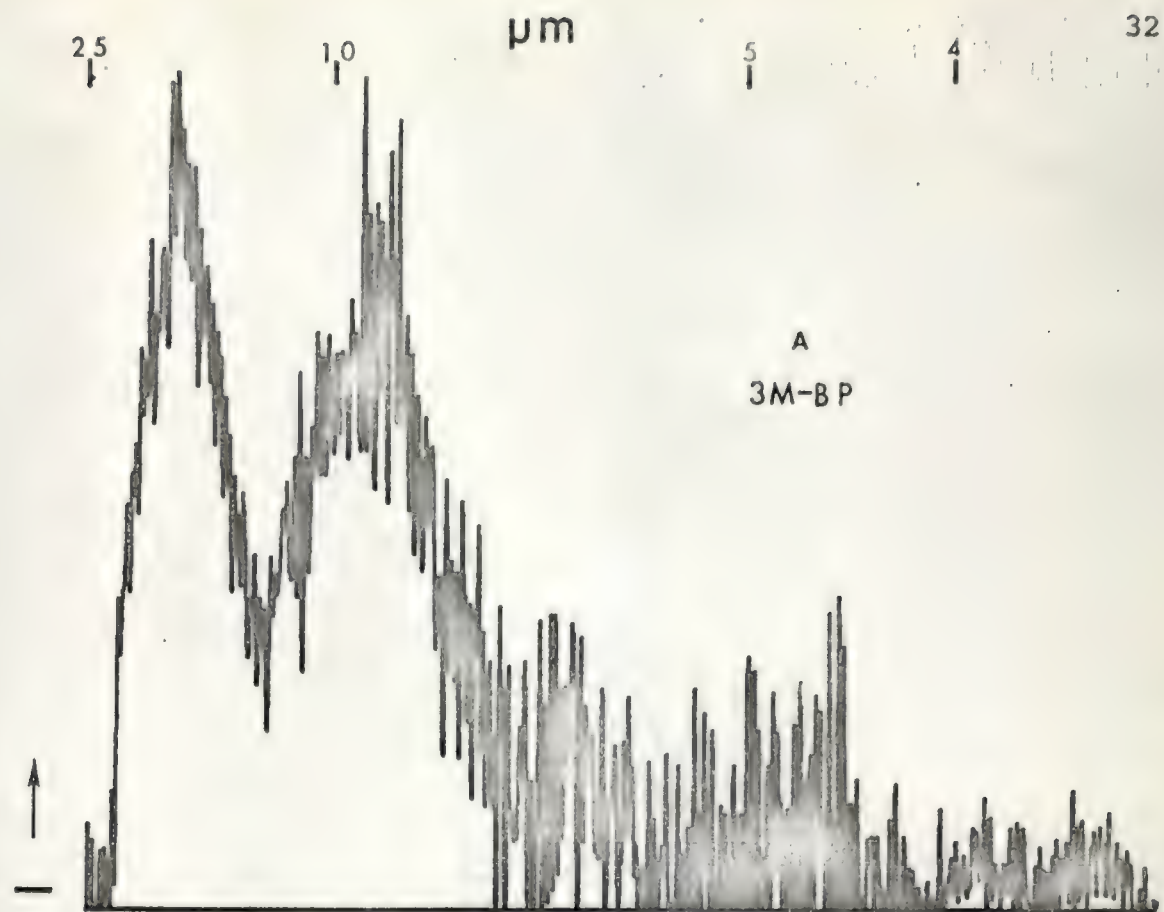
Straight arena experiments

The first set of experiments were run in a straight arena to test the ability of body lice to detect blackbody radiation and move towards the source of IR. An arena was made with plexiglass, 24 cm X 7 cm, with 1 cm high walls. The inner surfaces of the walls were of plexiglass which prevented the lice from climbing. The arena floor was made of white blotting paper, which was changed after each experiment. The arena was marked every 3 cm, forming 8 compartments or sections. Two 1 inch square, 5-watt tape heaters were covered with aluminum foil and were used as blackbodies. One side of each heater was painted with the 3M black paint. Each heater temperature was adjusted

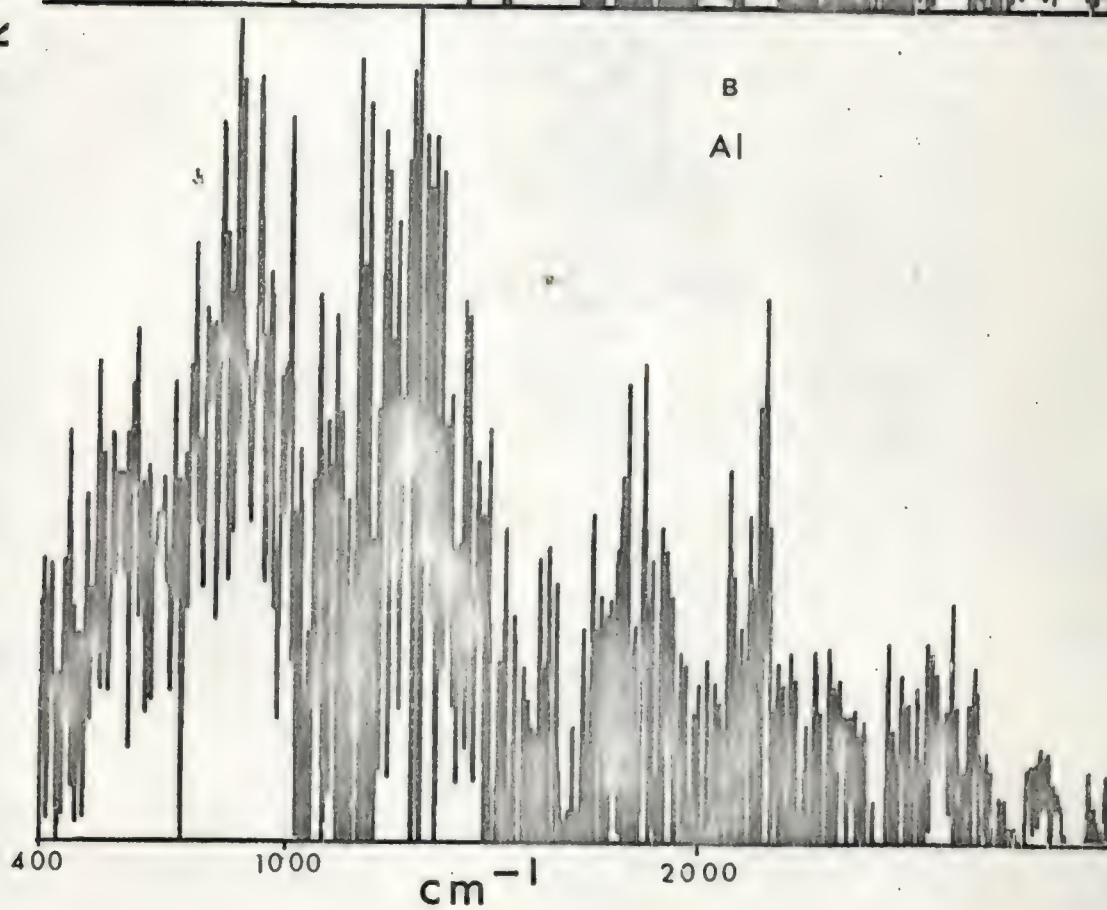
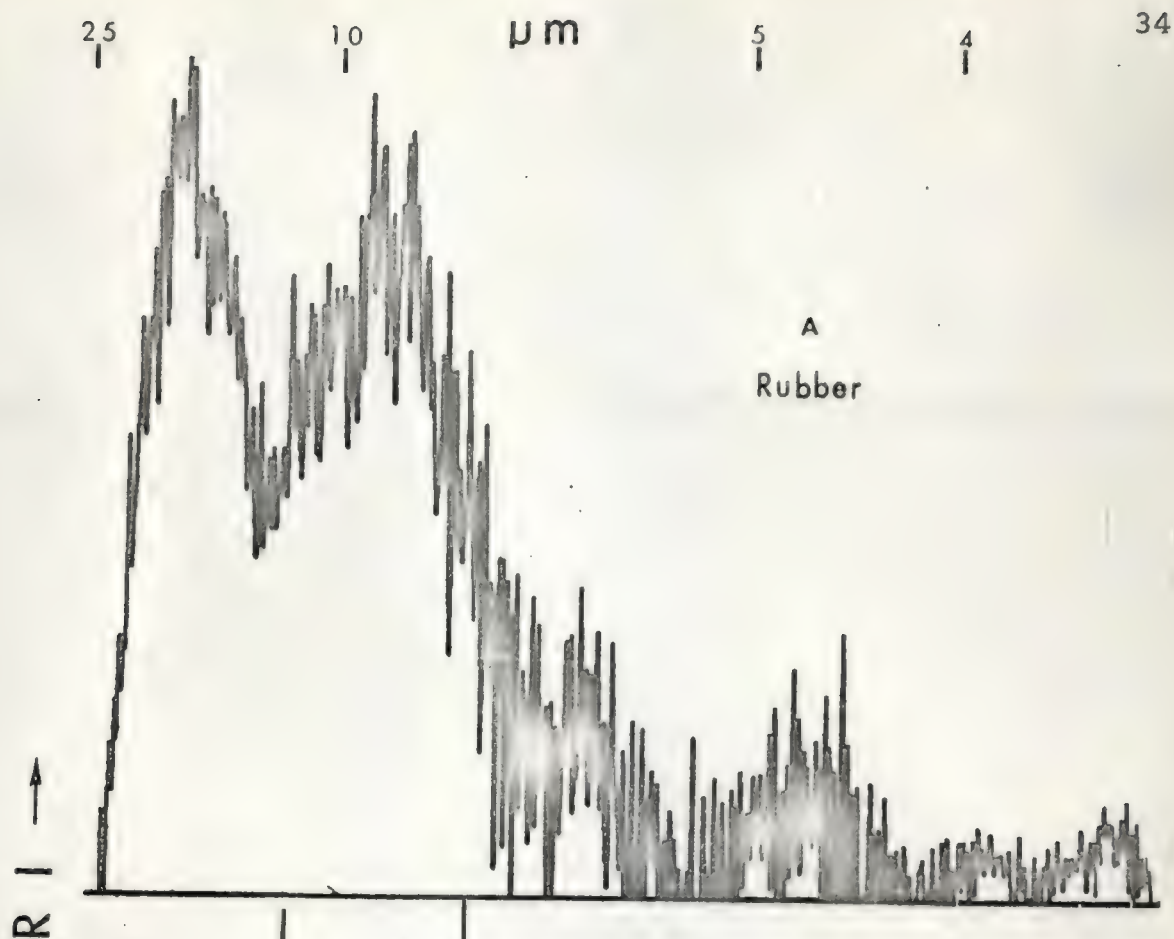
Figure 1. Infrared spectrum of human hand, in the range of 25.0 to 3.3 μm (400 to 3,000 cm^{-1}). The y-axis represents the radiation relative intensity (RI) and is not comparable to the other spectra. Spectrum was obtained in a FTS-14 Interferometer (Digilab-Block Engineering, Inc.).



Figures 2a, b. Infrared spectra of (a) black paint (Velvet coating no. 101-C10, emissivity = 0.97; Minnesota Mining and Manufacturing Co.) and (b) cellophane (cigarette package wrapping), heated to a 33 C surface temperature. Spectral range covers from 25.0 to 3.3 μm (400 to 3,000 cm^{-1}). The y-axis represents the radiation relative intensity (RI) and is not comparable to the other spectra. Spectra were obtained in a FTS -14 Interferometer (Digilab-Block Engineering, Inc.).



Figures 3a, b. Infrared spectra of (a) rubber glove (Surgeons glove, grade A; Perry Rubber Co.) and (b) aluminum foil, heated to a 33 C surface temperature. Spectral range covers from 25.0 to 3.3 μm (400 to 3,000 cm^{-1}). The y-axis represents the radiation relative intensity (RI) and is not comparable to the other spectra. The intensity readout gain for the aluminum was greatly increased over the other spectra, and the output is mainly electronic noise amplification. Spectra were obtained in a FTS-14 Interferometer (Digilab-Block Engineering, Inc.)



and maintained at 33 C using the temperature controller. Even though both of the surfaces of a heater (black and aluminum) were at 33 C, the black one irradiated more energy as IR than the aluminum, due to the black's higher emissivity, 0.97 (manufacturer's specifications) versus 0.10 (Hemmerdinger and Hemback, 1965).

The heaters were suspended freely at each end of the runway, 1 cm above the arena floor. The experiments consisted of having a blackbody radiator (hereafter called blackbody or BB) at one end and an aluminum one (hereafter called aluminum or Al) at the other. Fifty, one-day-starved lice were released on the center of the arena. At the end of 3 or 5 minutes the lights were turned on and the number of lice on each compartment was recorded. Controls were run with both heaters off. The arena compartments were enumerated or classified according to their relative position to the blackbody. Lice on the two central sections were not recorded, because it was hypothesized that a louse found on these sections had not reacted to the offered stimuli. The compartment closest to the blackbody was weighted with a factor of +3, the next ones, +2 and +1. The section closest to the aluminum was given a factor of -3, and the following, -2 and -1, respectively. This was done because a louse recorded at a 3 position is weighted statistically more than

one at 2 or 1; that is, a louse that had moved closer to a heater should be considered more than one at a 2 or 1 position. This method of weighting the data according to the position of the specimen has been used with data from several types of insect olfactometers (M. S. Mayer, personal communication, 1970). It was necessary to normalize the recorded values on base 100, because the number of responding lice in each experiment varied considerably. This normalization was carried on prior to weighting the data.

Round arena experiments

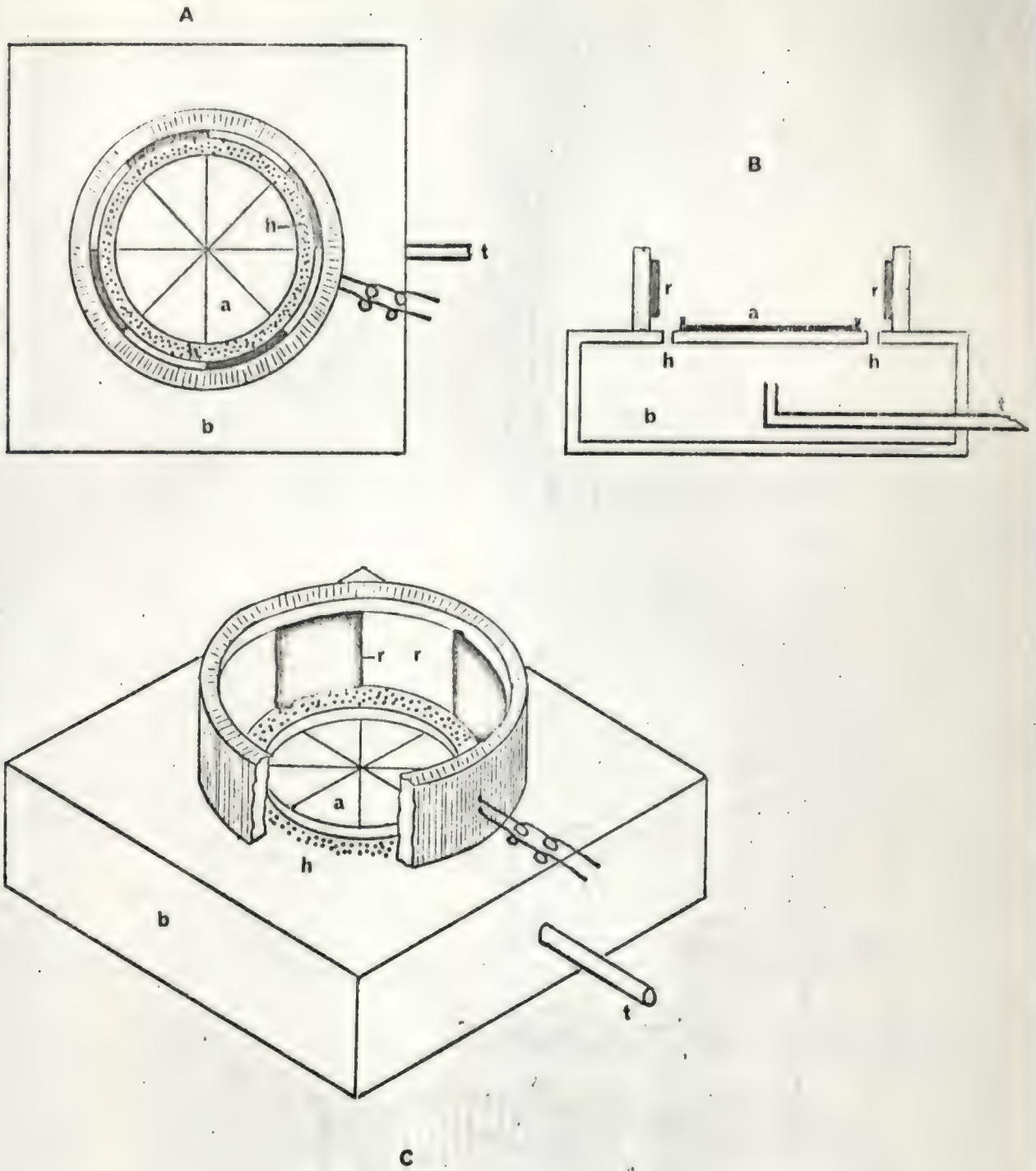
As previously stated, Wigglesworth (1941) believed that lice did not detect IR radiation; the response to the "warmth" of an object was due to the heated air, i.e., the convective heat. In order to determine whether lice could detect IR when convective heat was eliminated, the following experiment was conducted.

A circular arena with an 8 cm radius was made out of a flat plastic dish with a rim of 0.4 cm to prevent the lice from escaping. The arena was positioned on the center of the top of a 30 X 30 X 4 cm plexiglass box. Two circles, 8.0 and 9.0 cm radii, were traced on the box top and a maximum number of 1.5 mm holes drilled between them. A 60 X 2.5 cm tape heater was lined with aluminum foil and attached

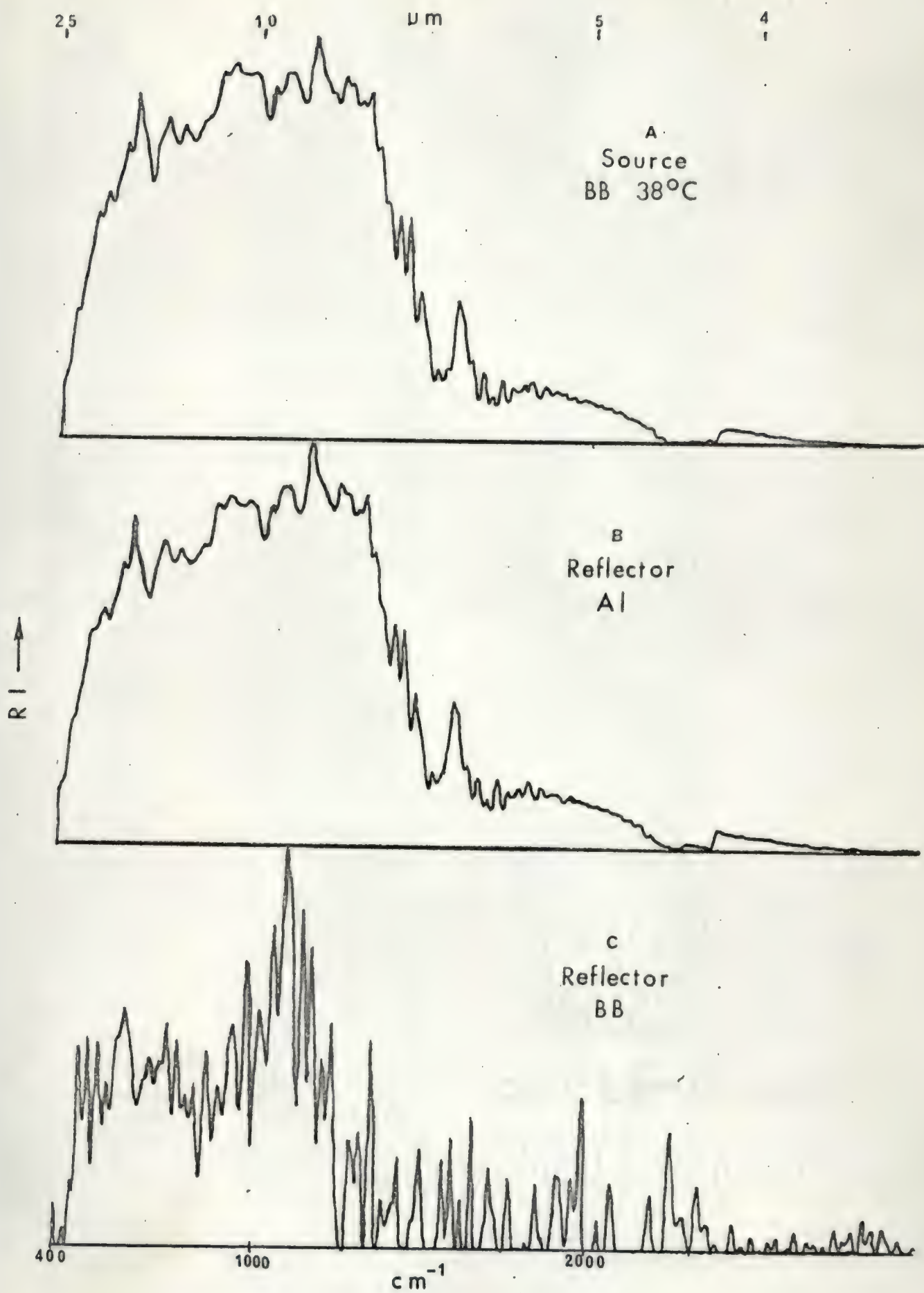
to the inner part of a plexiglass tube of 2.5 cm high and 9.25 cm inside radius. This ring was positioned on the box top (see Figures 4a, b, and c). The intention was to blow air into the box and force the air to escape through the small holes; the plexiglass tube (with the tape heater) and the arena walls would serve as channeling walls for the air. In this manner, a curtain of fast moving air was formed between the heater and the arena, eliminating the effect of convective heat. Compressed air from the laboratory line was used. It was cleaned and dried using the following attachments: 1) a pressure valve; 2) a water trap; and 3) a cylinder of glass wool followed by a second cylinder with activated charcoal, and finally calcium sulfate (Drierite[®]). The flow of air was maintained constant at $12,000 \text{ cc min}^{-1}$, as measured with a glass flowrator (Fischer and Porter, Co.). The air was introduced through the bottom of the box. The box was sized to prevent turbulence that might cause differential flow rates through the holes on the box top.

The tape heater was divided in 8 equal sections; half of them were painted with the 3M black paint and the rest left with plain aluminum (see Figures 4a and b). Thus, blackbody opposed blackbody, and aluminum opposed aluminum. This prevented the aluminum, an efficient reflector (see Figures 5a, b, and c), from reflecting the IR from the

Figures 4a, b, c. Round arena apparatus designed to study the lice detection of infrared radiation, but eliminating the convective heat arising from the blackbody and aluminum radiators (r). A vertical laminar air flow was provided between the radiators and the arena. A constant air flow of $12,000 \text{ cc min}^{-1}$ was supplied to the plexiglass box (b) through a tube (t). The arena (a) was positioned over the box and it was surrounded by the radiators. Air flowed from the box through holes drilled around (h) the arena. Apparatus drawings are not at scale.



Figures 5a, b, c. Infrared spectra of infrared radiation (a) reflected at a 90° angle from (b) aluminum and (c) black paint (Velvet coating no. 101-C10, emissivity = 0.97; Minnesota Mining and Manufacturing Co.), demonstrating the good reflectivity of aluminum and the poor one of black paint (reflectivity is inversely proportional to emissivity). Blackbody infrared source was a blackbody radiator (black paint) at 38 C surface temperature. The high temperature was used to provide enough energy for analysis after reflection. Spectral range covers from 25.0 to $3.3\ \mu\text{m}$ (400 to $3,000\ \text{cm}^{-1}$). The y-axis represents the radiation relative intensity (RI). The intensity readout gain for the blackbody was greatly increased over the other spectra. Spectra were obtained in a FTS-14 Interferometer (Digilab-Block Engineering, Inc.).



black surfaces (blackbodies). The tape heater was maintained at 33 C (surface temperature) using the temperature controller. The arena floor was made out of white blotting paper with a 7.5 cm radius so that the paper was 0.5 cm from the arena wall. The paper was divided (with pencil lines) into 8 equal partitions (pie wedges) with 4 diameter lines; a 5.5 cm radius circle was also traced on the paper. The paper was changed after each experiment. One hundred, one-day-starved lice were released in the dark on the center of the arena. After 5 minutes, the lights were turned on and the number of lice on each compartment counted with a hand counter. Only those lice that reached the 5.5 cm radius circle were recorded as responding. This outer circle represented 46 percent of the area of the arena, and was 3.75 cm from the heater surface.

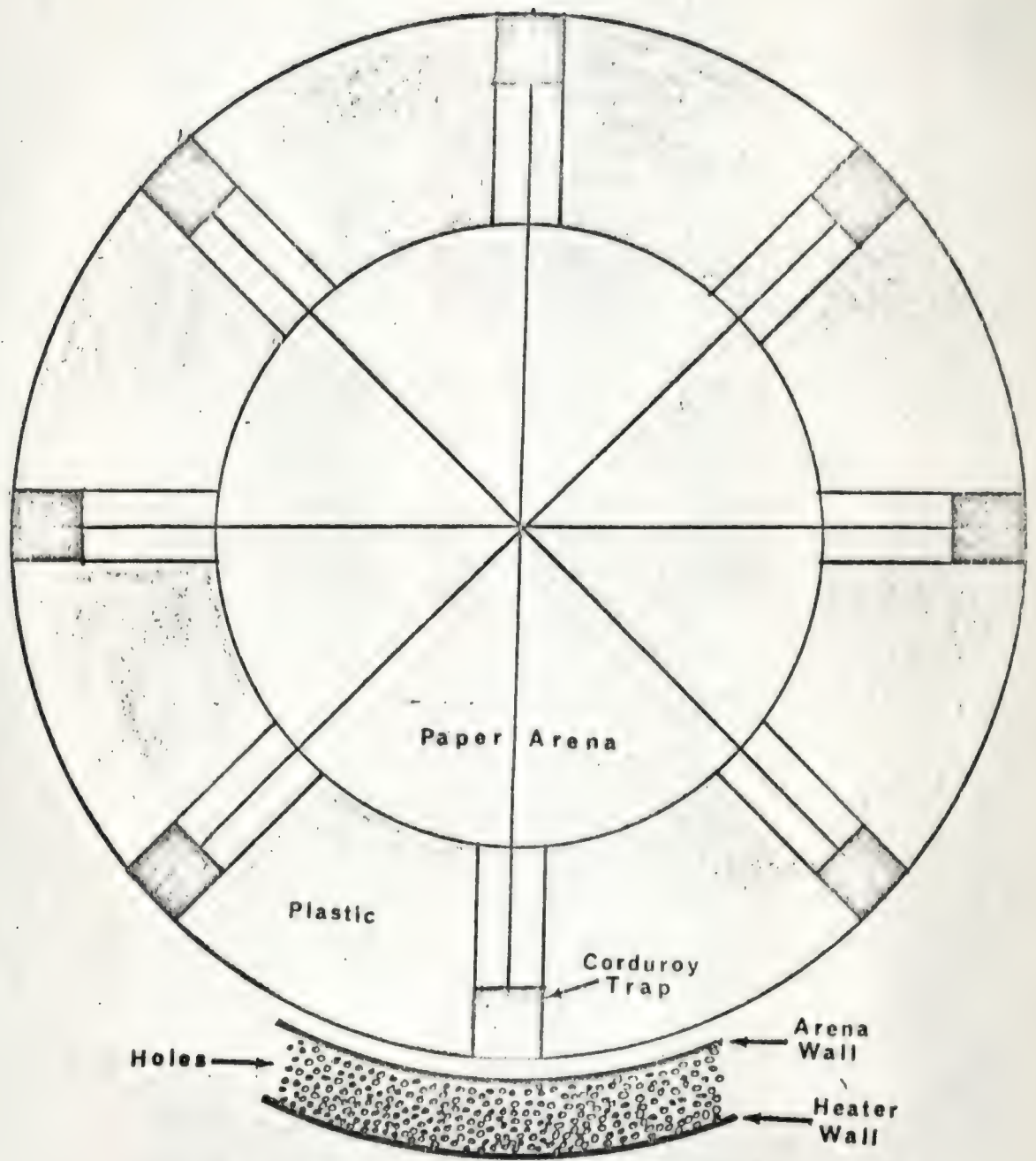
This experiment posed the following problem: Did the lice discriminate between the two heated surfaces (black or aluminum) after they reached the arena border, or did they orient themselves before reaching that point? In order to investigate the above, the arena floor was modified in the following manner: circles of varied radii, 2.0, 4.5 and 6.0 cm were traced on the paper arena. The arena was again divided into 8 equal compartments by the 4 diameter lines. It was then cut along the circle (i.e., the 6.0 cm radius)

but leaving a 1 cm wide strip along the diameter lines. This way 8 strips of paper extended from the arena, and each pointed toward a different section of the tape heater (Figure 6). The same procedure was followed with the 4.5 and 2.0 cm circles. The reason for the different strip-lengths was to determine how far away lice could detect and orient to the blackbodies. One cm square pieces of black corduroy were attached to the end of each strip and this served as a "trap" for the lice. Lice prefer rougher surface, hence, they prefer cloth over paper. These patches prevented the lice from moving away from the strips once they had made their choice. One hundred lice were released on the center and after 5 minutes the lice on the patches were counted as responding lice. The values obtained were normalized to base 100 because not all the lice responded. Each test was replicated five times and the number of lice per compartment was recorded individually and analyzed by a "t" test.

CO₂ monitoring experiments

The response of body lice to blackbody radiation was quantified by monitoring constantly the CO₂ output before and during IR stimulation. In this indirect method, it was assumed that CO₂ output was proportional to locomotive

Figure 6. A schematic diagram of the 2.5 cm strip arena. It illustrates the arrangement of the strips and the corduroy traps, in relation to the whole arena and the heaters. Similar format was followed in making the 4.75 and the 7.5 cm strip arena. Schematic diagram is drawn on a 1:1 scale.



activity. It had been observed that akinetic lice began moving when a warm object was brought close to them (Homp, 1938; Howlett, 1917). Undoubtedly, this activity was difficult to measure and quantify. Turner and Charity (1971) described a method for measuring moth activity by monitoring the CO_2 output.

The lice were confined in a 4.0 cm high chamber made from a 3.8 cm diameter plexiglass tubing, and mounted on a 8 cm square plexiglass plate. Two 0.5 cm diameter and 3 cm long plexiglass tubes were attached 1.0 cm high on opposing sides of the chamber for the input and output of air. The chamber top was a removable 8 cm square plexiglass plate. Two 10 K Ω , 2-watt resistors were suspended horizontally from the chamber top; when the top was positioned, these resistors were 2 cm from the arena floor. The resistors were 0.8 cm apart and each connected to a different temperature controller set at 33 C surface temperature (see Figure 7). Both resistors were covered with aluminum foil, and one of them painted with the 3M black paint to serve as a blackbody radiator. A thermistor, within the tip of a hypodermic needle, was vertically inserted between the ends of the resistor and the chamber top and positioned to have its tip equidistant from both resistors and 0.3 cm above the chamber floor. This thermistor was positioned so to

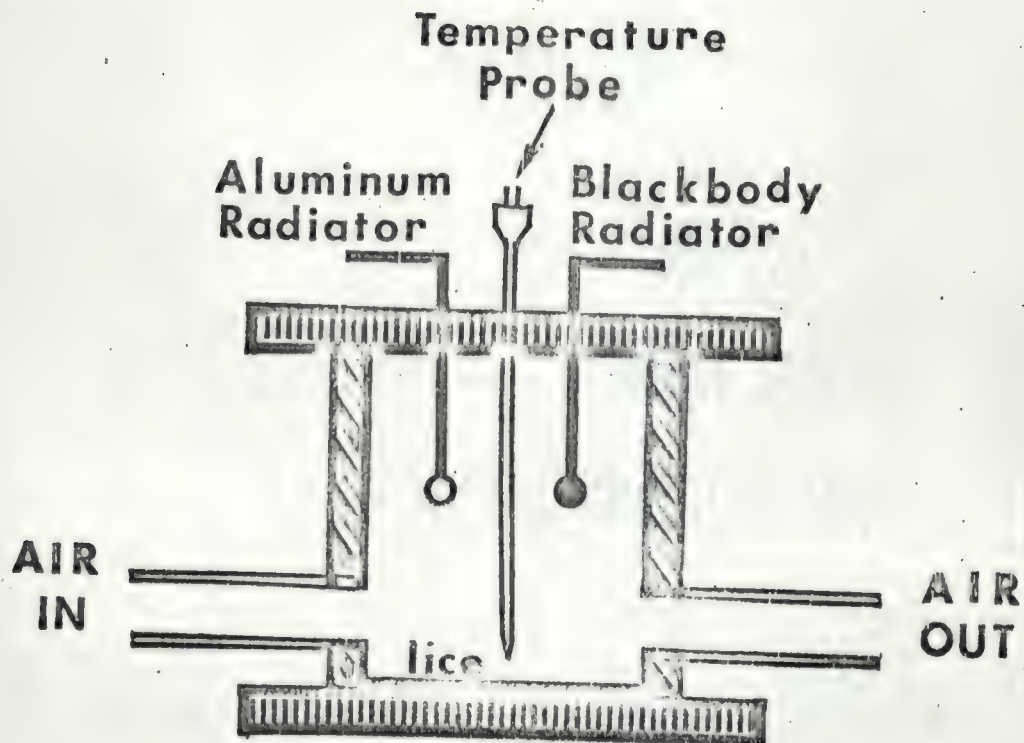
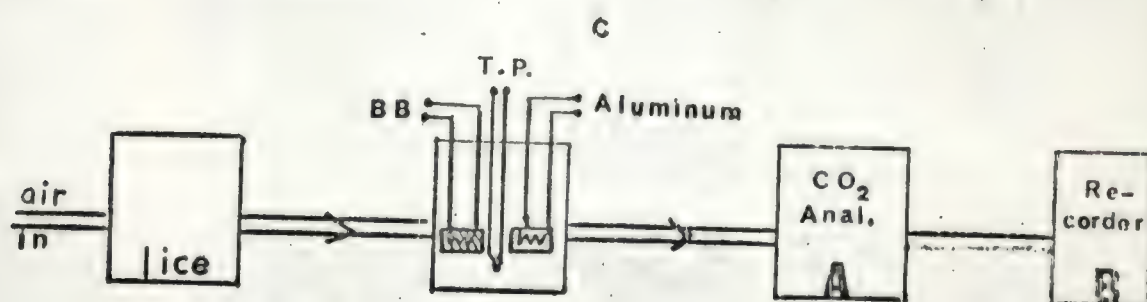
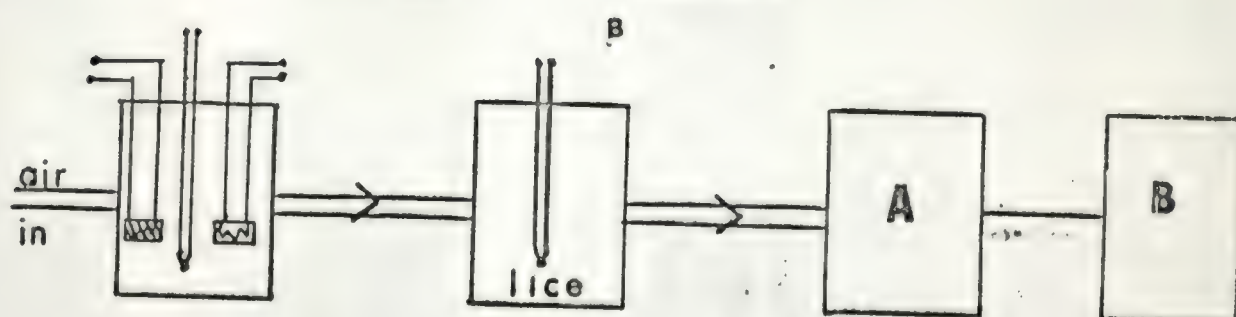
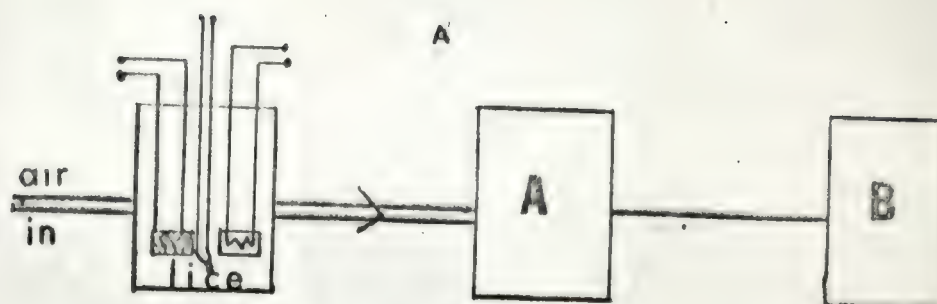


Figure 7. CO₂ chamber for monitoring lice activity during stimulation with blackbody infrared radiation. The chamber walls were made of plexiglass and the chamber floor of blotting paper. Each radiator was independently connected to a temperature controller, and the temperature was monitored using the temperature probe and a digital thermometer. The chamber was connected to a compressed air line (air in) and to the CO₂ analyzer (air out). Schematic diagram not drawn at scale.

measure the air temperature. It was connected to a digital thermometer.

The chamber floor was made of blotting paper. The chamber was connected with Tygon[®] tubing to a CO₂ analyzer (IR analyzer, LIRA model 200, Mine Safety Appliances, Co.). Two lines supplied air from a compressed air tank, at a constant rate, to the analyzer. One of the two lines was connected directly to the analyzer and served as a reference; the air from the other line passed through the chamber with the insects. The difference in the concentration of CO₂ between the two lines could be read directly in parts per million (ppm) from a metered scale or plotted on a time basis. CO₂ concentration was recorded by a Leeds and Northrup, Speedomax strip-chart recorder (see Figure 8a). Preliminary tests indicated that as few as 10 akinetic lice gave a reading of about 5 ppm. It was decided that 50 lice would give more meaningful and statistically valid readings. Preliminary tests with 50 lice indicated that the CO₂ concentration increased between 30 and 60 percent when only 20 lice were walking, upon stimulation by the blackbody radiation; this was observed under well-lighted conditions, but the rest of the experiments were conducted in total darkness by placing the chamber inside a closed 0.60 X 0.60 X 0.75 m box. This box was kept at a tempera-

Figures 8a, b, c. CO₂ monitoring flow chart. (a) Lice were held in the same chamber with the heaters. Air flowed from the chamber to the CO₂ analyzer (A). The output from the analyzer was recorded on a strip chart recorder (B). (b) Control experiments in which the lice were held in a chamber following the heaters chamber. (c) Control experiments in which the lice were held in a chamber preceding the heaters chamber. A further control experimental setup (not illustrated) consisted of removing the lice altogether from the system.



ture of about 26 C by way of an air heater connected to a temperature controller. Air flow to the lice chamber was calibrated to 50 cc min^{-1} and the line air kept at a temperature close to 26 C.

During any given test, the lice were introduced into the chamber connected to the sample line. The box was closed and 30 minutes were allowed for the lice to go into akinesis, before any test was conducted. The heaters were turned on and off, one at a time according to the test. In one series of tests, the blackbody was turned on for 2.5 minutes, off for 2.5 minutes, on for 2.5 minutes, and so on. In another series, this procedure was repeated with the aluminum heater. In another, the blackbody and aluminum heaters were alternated after an off period. Several control experiments were run, as follows: a) Lice removed from the chamber, b) lice in a container next on the line from the heater's chamber (heaters on) (see Figure 8b), c) lice in a container preceding the heater's chamber (heaters on) (see Figure 8c). Variation in CO_2 output over a long period of time was determined by recording the CO_2 output every 5 minutes for 6 hours.

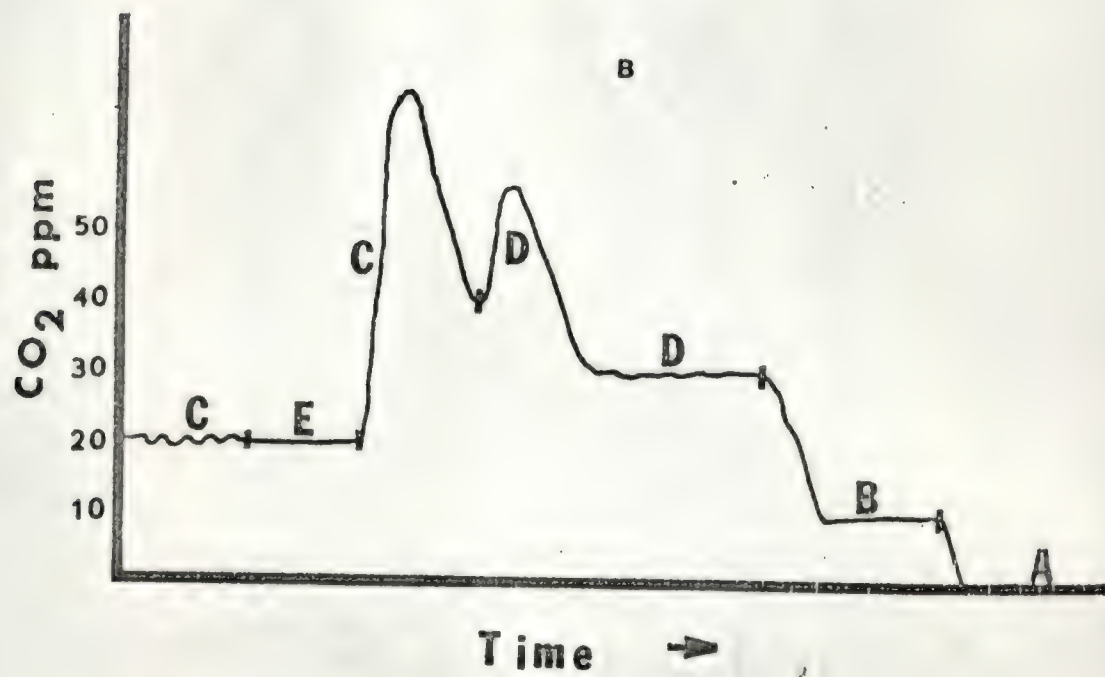
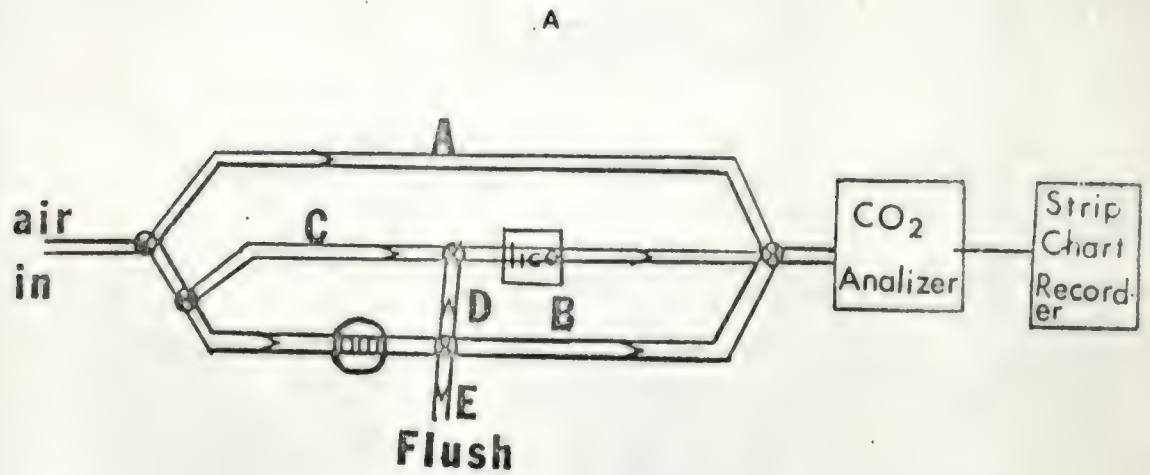
In order to test the effect of odors and CO_2 emanating from human skin, air was blown through a chamber attached to the arm of volunteers (K. F. and M. S.). Air drawn

through this chamber was introduced in a chamber with 50 lice and their CO_2 output determined. Recording the results, from these experiments presented several problems, because a baseline of the lice CO_2 output had to be determined prior to introducing the air from the skin chamber. But the air from the skin chamber had accumulated high concentrations of CO_2 and drove the instrument off scale. So a procedure was devised by which the air from the skin chamber was first flushed, then the air from the lice chamber (since it had accumulated CO_2 meantime) and finally the two chambers were connected together. At the end of an observation the skin chamber was disconnected and the lice CO_2 output allowed to attain baseline conditions again. The CO_2 output by the skin of the arm was also measured by connecting the chamber directly to the CO_2 analyzer (see Figures 9a and b).

Reactions to an artificial finger and varied stimuli

The lice were observed walking on an arena floor of arithmetic graph paper and their "tracks" recorded on a similar paper. The arena was surrounded by white paper walls and a lamp suspended overhead to diminish the problem of differential illumination previously discussed. Individual lice were stimulated using an artificial finger (hereafter called "finger"). A 10 K Ω , 2-watt resistor was

Figure 9a, b. (a) Flow chart for monitoring the CO₂ output of lice during stimulation with human skin gaseous emanations. The air could flow through five different paths: A, directly to the analyzer for reference; B, through the arm chamber; C, through the lice chamber; D, through both arm and lice chambers; and E, flushed out of the system after passing through the arm chamber. (b) Diagrammatic representation of the CO₂ levels in the system under the different pathways air flowed through. Time is not scaled. See text for more explanations.



covered with molding clay and this inserted into a finger of a rubber glove; this finger had a final diameter of 1 cm. The blackbody radiation output of the rubber glove is very similar to human skin. The resistor functioned as a heater and it was wired to a temperature controller which was adjusted for a 33 C surface temperature. Lice were made to follow the "finger" or the "finger" was brought close to the path of walking lice to see how they would react to it.

In another arena designed to eliminate the convective heat effect, the arena floor was made of fine ninon (fine nylon fabric) stretched over a 10 cm diameter container, to the bottom of which clean, dried air was supplied at the rate of 12,000 cm min⁻¹. Reactions of walking lice to the "finger" were also studied in this arena.

Several unsuccessful attempts were made at sealing the pegs on the tip of the antennae with different kinds of glues, adhesives, paint, etc. Most of the time the material did not stick or if it did, it peeled off when dry. Cutting the antennae failed because the great amount of bleeding was considered harmful; sealing the cut end also failed. The pegs were finally removed by squeezing the extreme tip of the antennae with a very sharp pair of tweezers while the lice were anesthetized with CO₂. Twenty-four hours after this treatment the lice were allowed to feed on the

investigator's arm to compare their feeding behavior with normal lice. They were also stimulated with the "finger."

Morphological studies of the antenna

Body lice antennae were studied using the light microscope and the transmission and scanning electron microscopes. The studies concentrated on the pore and tuft organs situated on the fourth and fifth segments, and the pegs sensillae on the fifth.

Light microscopy

Antennae from 1st, 2nd, and 3rd instar larvae and adult lice were cut off and mounted on glass slides with a clearing mounting media (CMC-10, General Biological Supply House, Inc.) or resin (preservaslide, 60 percent resin in xylene) for whole mount studies. The number of tactile hairs, tufts and pegs present on the antennae of each instar were recorded. Adult lice from the Burundi, Freetown and Korean colonies were prepared in a similar manner. Preserved specimens of body and head lice (P.h. capitis) loaned by the U. S. National Museum, Washington, D. C., through Dr. R. I. Sailer, USDA, ARS, ENT, IIPB Branch, and specimens from the previously mentioned colonies were studied regarding the number of pegs and tufts on the antennae. This was done as a means of further documenting

the discrepancy in the number of tuft organs as stated by Wigglesworth (1941) and by Miller (1969).

The crystal violet technique (Slifer, 1960) for identifying receptors with openings where nerve endings are exposed to the air was used with fresh adult antennae. This possibility of permeability of the walls of the basiconic pegs was also investigated using aniline blue instead of crystal violet (P. S. Callahan, personal communication, 1970). The pressure technique used by Slifer, Prestage and Beams (1957) to locate open tip thick-walled sensors was applied to fresh antennae. Other antennae were embedded in paraffin and sectioned at 5 or 7 μm . These sections were stained with Mallory's triple stain. Thick, 2 μm sections of epoxy-embedded antennae for transmission electron microscopy (see next section) were cut with the ultramicrotome using glass knives and stained with Safranin O, Toluidine Blue and Auromine O (R. M. Ropell and T. Carlysle, personal communication, 1970). Examination of material was performed using a Zeiss photomicroscope II, under phase contrast or Nomarski differential interference contrast. Microscopic measurements were made using an eyepiece micrometer.

Transmission electron microscopy

Antennae from live lice were fixed for 3 hours in

phosphate buffered 5 percent gluteraldehyde, washed in buffer, osmicated for 4 hours in 1 percent OsO_4 (osmium tetroxide), washed, carried through a dehydrating ethanol series, then placed in propylene oxide and embedded in an epon-araldite mixture (Mollenhauer, 1964). Ultrathin sections were cut with a diamond knife on a Sorvall MT-2 ultramicrotome and picked up with Formvar-coated copper grids. They were stained with saturated uranyl acetate and lead citrate (Venable and Coggeshall, 1965). Examination of sections was carried out on a Hitachi 125-E electron microscope at accelerating voltages of 50 or 75 KV.

Scanning electron microscopy

Lice heads were fixed in 5 percent glutaraldehyde. Some specimens were post-fixed in 1 percent OsO_4 . Some of these post-fixed heads were sonicated in a Sonicator (Ladd Research Industries, Inc.) to cause breakage of some of the pegs, since it had been observed that osmic fixation caused them to become very brittle. The heads were mounted on specimen stubs with a silver base paint; some of them were coated with 200-300 Å gold or gold-palladium in a Denton DV-502 high vacuum evaporator, others were examined with no coating. Examination was done with a Cambridge Mark II A Stereoscan Microscope at operating voltages of 5-20 KV.

CHAPTER IV

RESULTS AND DISCUSSIONS

Straight arena experiments

The results from the straight arena clearly indicated that lice responded selectively to the blackbody. Mean values of percent-responding lice per compartment are summarized in Table 1. The percent and their weighted values for the blackbody and aluminum sides of the arena are shown in Table 2, together with the "t" statistic for comparison between the two sides. The records of lice on the blackbody side were significantly higher (0.01 level) than on the aluminum, at both times tested, 5 and 3 minutes. No difference was obtained in the control experiments, that is, while the heaters were off. Weighting the raw data, according to the relative position of the compartment, lowered the values, but did not affect the significance of the results.

The number of lice "responding" when the heaters were on was greater (highly significant) than during control experiments (Table 3). The 2-minute difference between

Table 1

Percent of the total number of responding lice
recorded at each compartment in the straight arena.
Eight replications per experiment.

Compartment	Heaters on		Control	
	\bar{x}	Sx	\bar{x}	Sx
5 Min.				
+3	31.5	8.54	15.2	10.26
+2	16.2	4.64	11.4	13.53
+1	12.8	8.12	24.0	20.45
-3	16.3	7.49	13.0	7.51
-2	13.5	7.23	18.2	5.84
-1	9.7	5.94	19.2	7.93
3 Min.				
+3	39.2	13.42	23.1	16.33
+2	17.2	6.26	9.1	11.43
+1	8.8	4.49	16.5	14.42
-3	19.8	12.27	19.5	15.36
-2	8.8	6.59	23.7	7.31
-1	7.2	4.35	9.1	9.09

Table 2

Percent of the responding lice (N) moving toward the blackbody and aluminum heaters in the straight arena. Weighted values of the results (W) are also shown. Results are of 8 replications.

Time		Heaters on				"t"	Control				"t"
		BB		Al			BB		Al		
		\bar{x}	Sx	\bar{x}	Sx		\bar{x}	Sx	\bar{x}	Sx	
5 Min	N	60.5	6.85	39.5	8.15	6.26**	49.8	3.88	50.2	3.60	.23 N.S.
	W	139.6	21.75	85.0	23.58	5.38**	90.5	29.38	94.0	16.24	.25 N.S.
3 Min	N	65.0	11.31	35.0	11.17	4.64**	48.0	14.35	52.0	14.47	.44 N.S.
	W	160.3	33.48	82.8	30.26	4.20**	97.5	29.56	114.0	40.47	.80 N.S.

**Highly significant at a 0.01 level.

N.S. Not significant at 0.10 level.

Table 3

Average number of lice (out of 50) responding when the heaters were on or off (control) in the straight arena. Results are of 8 replications each.

Time	on	off	"t"
5 Min	36.8	17.5	5.47**
3 Min	32.2	13.2	3.24**
"t"	.91 N.S.	1.27 N.S.	

**Highly significant at a 0.01 level.

N.S. Not significant at a 0.10 level.

the 3-and 5-minute experiments did not make any difference between the recorded number of responding lice. Perhaps, if shorter times had been used, a difference would have appeared. Wigglesworth (1941) reported that lice moved at an average speed of 27.2 cm min^{-1} at 21 C and the speed increased to 37.5 cm min^{-1} at 29C. Since my experiments were conducted at 22 C a speed of about 28 cm min^{-1} can be assumed. A louse released in the center of the arena facing toward the aluminum side would keep moving in that general direction (discussed in the Review of Literature). At the end of the arena or compartment -3 it would continue along the walls because of its strong thigmotaxis and thus move toward the blackbody side. Upon perceiving the stimulus on this side it would remain there. The total distance of 36 cm in a straight course could be easily covered in the 3 minutes. This would explain the greater number of lice recorded at +3 than at any other compartment. This experiment did not demonstrate that lice can detect IR from 12 cm away, as it may appear. As discussed in the Review of Literature, it has been reported that some blood-sucking insects can detect the hosts "warmth" from only 4-5 cm away (see next section).

Even though the heaters were above the arena the convective heat arising from them may still have affected the

lice. These experiments did not eliminate the convective heat as a factor, as will be seen in the next section.

Round arena experiments

The round arena experiments studied the alleged role of convective heat on the detection of the host's warmth by blood-sucking insects. The curtain of moving air (about 220 cm min^{-1}) eliminated the convective heat arising from the walls of the tape heater and its effect on the lice. The percentages of the lice recorded beyond the 5.5 cm radius circle (3.25 cm from the heater surface) in front of the blackbody strips were higher than the percentages in front of the aluminum strips. A "t" test demonstrated that the difference was significant at confidence levels higher than 99 percent (Table 4). Control experiments, that is, with the heater off, did not show any difference between the BB and Al areas, even at the confidence levels of 0.50. By eliminating the convective heat, these results clearly demonstrated that lice were not reacting to the convective heat, as proposed by Wigglesworth (1941), but to the total IR output of the walls of the heater. The number of lice considered to have responded when the heater was on was significantly greater (0.01 level) compared to the number when it was off. This is explained by the observation that lice become more active when close to an IR source.

Table 4

Percent of responding lice recorded in front of heated areas at 33 C in the round arena. Averages of 5 tests per arena type and 4 replications per arena.

	HEATER ON				HEATER OFF						
	BB		Al		BB		Al				
	\bar{x}	Sx	\bar{x}	Sx	\bar{x}	Sx	\bar{x}	Sx			
ARENA (a)											
Solid	17.80	8.02	6.83	3.38	6.09***		12.85	8.07	12.20	4.72	0.31 N.S.
1.5 cm strip	15.25	6.14	9.55	3.24	3.67***		13.15	4.77	11.40	6.20	1.00 N.S.
3.0 cm strip	12.85	6.53	12.25	5.39	0.32 N.S.		11.35	5.73	13.55	7.28	1.06 N.S.
5.5 cm strip	12.05	6.18	12.70	5.52	0.35 N.S.		13.20	3.75	11.90	5.67	0.85 N.S.

(a) Arena types were solid, the 6.0 cm (3.25 cm from heater surface; 4.5 cm (4.75 cm from heater surface) and 2.0 cm (7.25 cm from heater surface).

***Highly significant at 0.001 level.

N.S. Not significant at 0.10 level.

Results from the experiments with the arena cut into strips of different lengths and facing each heater area demonstrated that lice could detect the difference in IR output between BB and A1 at distances of 3.25 cm, but not at 4.75 or 7.5 cm. The 1.5, 3.0 and 55 cm strips were detached from the solid center of the arena at distances of 3.25, 4.75 and 7.5 cm from the heater surface, respectively. Therefore, these were the distances from the IR source at which a louse left the arena center area and entered one of the 1 cm-wide strips and was recorded as "responding." The louse was committed to a particular strip at this distance from the heater. Since approximately equal numbers were recorded on strips facing A1 and BB, it can be assumed that when the IR stimulus was absent the lice moved at random (see Table 4). In the case of the 6.0 cm arena (1.5 cm strip) the lice may have outwardly dispersed at random. Those lice that walked right into one of the strips may have kept moving straight until reaching the corduroy "trap." But a louse reaching the arena edge, in between strips, moved along this edge (because of its strong thigmotaxis) until it encountered a strip and followed the edge of the strip. It is this group of lice reaching the arena edge that makes the choice of strip, i.e., either moving toward the IR source and therefore following the

arena edge until reaching a strip, or moving toward the A1 side and being "trapped" at the end of that strip.

The number of lice that "responded" with the heater on was greater in the 1.5 cm strip experiment (0.10 level) as seen on Table 5. No difference was observed between the number responding in the 3.0 cm and 5.5 cm strips experiments. This further substantiates the fact that lice detected IR at 3.25 cm away but not a 4.75 or 7.5 cm.

CO₂ monitoring experiments

These experiments were designed to quantify the observed response of lice to blackbody IR radiation stimulation as an increase in their locomotive activity. Quantification was possible by equating locomotive activity to CO₂ output of 50, one-day-starved adult lice. The major problem encountered during these experiments was that CO₂ production by the same number of lice, with no stimulus offered, varied considerably, and thus prevented pooling the data of repeated experiments. The data were pooled in cases in which the CO₂ concentration baselines were the same for two different sets of 50 lice from the same rearing batch.

When two groups of 50 lice were maintained for 6 hours with no stimulus under similar conditions and their CO₂ output recorded every 5 minutes, they had CO₂ baselines of

Table 5

Percent of lice recorded as responding to IR stimulation in the round arena. Results of 5 tests per arena type, 4 replications per arena.

Arena	Heater on		Heater off		"t"
	\bar{x}	Sx	\bar{x}	Sx	
Solid	86.80	6.76	58.20	14.84	3.92**
1.5 cm strip	65.6	12.97	45.2	17.77	2.07*
3.0 cm strip	67.20	19.93	57.80	10.78	0.93 N.S.
5.5 cm strip	71.60	15.90	80.00	16.60	0.82 N.S.

* Significant at a 0.05 level.

**Highly significant at a 0.01 level.

N.S. Not significant at a 0.10 level.

21.0 and 23.5 ppm. The standard deviations were 1.15 and 1.08 ppm, respectively. There was very little variation in CO₂ output over a long period of time, when conditions were constant. Baselines obtained in subsequent experiments ranged from 20.0 to 42.0 ppm. Several types of control experiments (see Figures 8b and c) demonstrated that the increase in CO₂ was not an artifact of the experimental arrangement. When no lice were in the chamber with the heaters and these heaters were on, no increase of the CO₂ above the zero line was observed; not even in cases in which both heaters, BB and A1, were turned on at the same time, demonstrating that the CO₂ differential was produced by the lice. In other experiments the lice were held in a chamber preceding the one with the heaters, and the heaters were turned on (see Figure 8c). No increase in the CO₂ concentration above the baseline was observed demonstrating that any increase in the CO₂ reading was not due to heating of CO₂ by the heaters or that the CO₂ analyzer was reacting to the small increase in the air temperature inside the heater chamber. I do not know if this temperature change remained after the air had passed through the narrow air hose and reached the CO₂ analyzer. In another control experiment, which consisted of reversing the chamber order, that is, the heaters preceding the lice chamber (see

Figure 8b), no change in CO_2 above baseline resulted. This proved that the lice were not reacting to any effect the heaters may have had on the system's air. The ideal control would have been to heat the air before it entered the lice chamber, but several attempts to do this using different types of heaters were unsuccessful, since the air was in a closed system and moving at a fast flow rate of 7.007 m min^{-1} ; no significant heating was obtained. However, it is shown later that the air temperature within the lice chamber did not influence the lice output of CO_2 , i.e., the lice kinetic activity.

Results from the experimental condition when two sets of 50 lice were subjected to heating of both the BB and A1 heaters are summarized in Table 6 (see Figures 10a and 10b). These values represent averages of readings taken every 2.5 minutes while either the BB or the A1 heaters were on for 65 min (60 min were allowed between heating). The data were pooled because the lice sets had equal CO_2 baselines, 22.0 ppm.

The difference between the CO_2 output when the BB was constantly on and when the A1 was on was highly significant (0.01 level). The temperature inside the chamber was raised by the same amount when either the A1 and BB were on. Therefore, the lice reaction was not due to a temperature

Table 6

Chamber temperature and CO₂ concentration when
BB or A1 heaters were maintained at 33 C for
65 min. Fifty lice in the chamber.

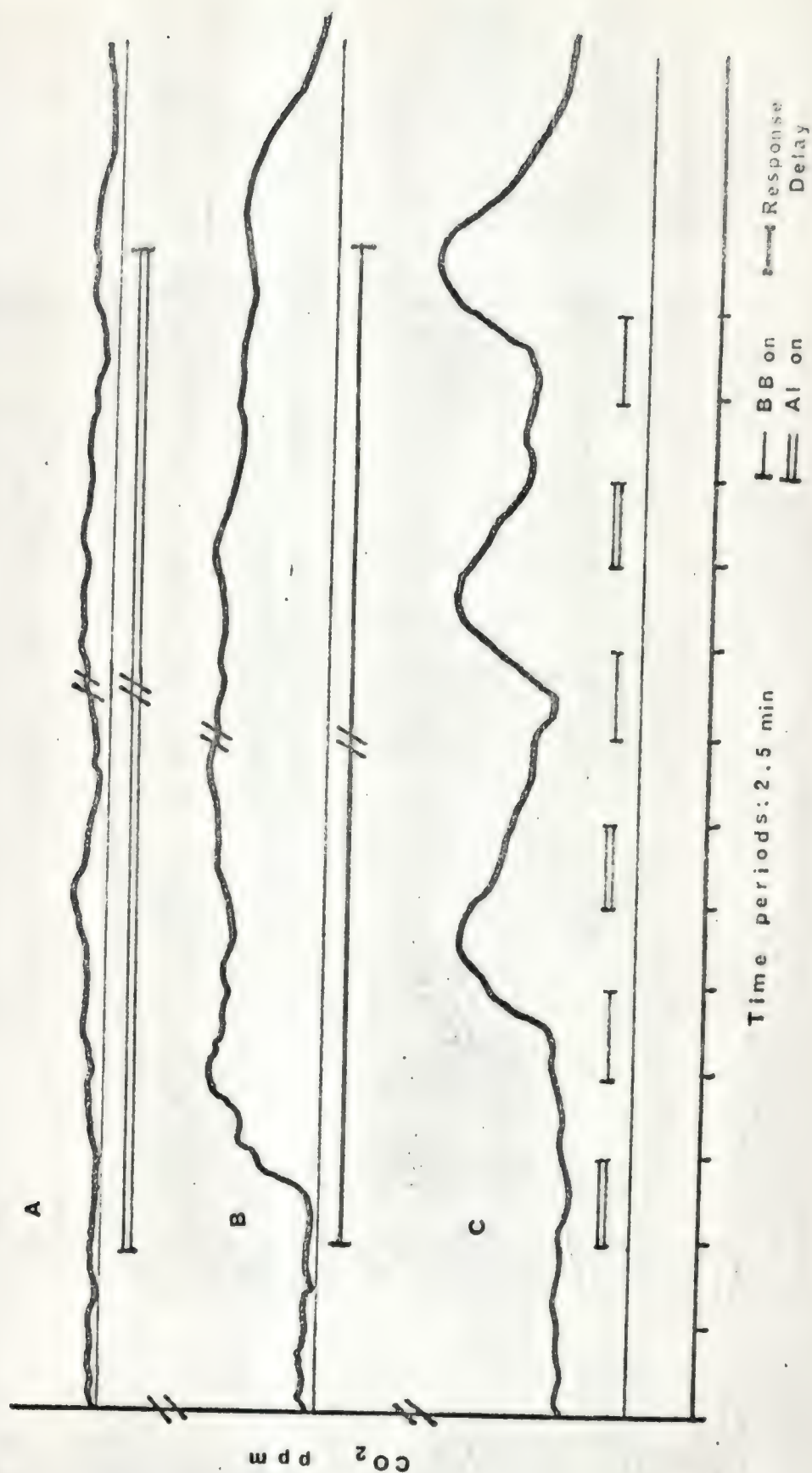
Heater	Temperature (C) (a)			CO ₂ (ppm)		
	\bar{x}	Sx	"t"	\bar{x}	Sx	"t"
BB	28.23	0.197	0.409 N.S.	38.38	2.35	22.38**
A1	28.21	0.224		25.24	2.24	

(a) Temperature during off period was 27.22 C.

**Highly significant difference at a 0.01 level.

N.S. Not significant difference at a 0.10 level.

Figures 10a, b, c. CO₂ output of lice during constant stimulation under (a) aluminum and (b) blackbody radiators, or (c) when both heaters were alternately turned on and off with a 2.5 min off period inbetween. Response delay represents the inherent lag on the system response due to air flow from the lice chamber to the CO₂ analyzer; it was approximately 1.1 min.



change, but to the differential output of IR from the BB.

The next experiments tested the effect of BB or A1 heating in short intervals on lice activity. For this, the heaters were turned on and off for periods of 2.5 min, one heater at a time (see Figures 11a and b) and letting one hour elapse before testing the other heater. Lice from the same rearing batch were used and their data pooled because of their similar CO₂ baseline, 41.5 ppm (Table 7). Neither the temperature at time-off (T-off) nor the change in temperature (Δt) varied between the A1 and BB stimulation, but the CO₂ concentration at time-off (CO₂-off) and the change in CO₂ concentration (Δ CO₂) was highly significant (Table 7). The CO₂ concentration during A1 heating did not increase above the CO₂ baseline limits.

One fact, not shown in Table 7, is that the initial increase in CO₂ concentration when the BB was turned on for the first time was the greatest CO₂ change (Δ CO₂) and subsequent changes due to BB IR stimulation were smaller; less than one-half of the initial rise (see Figure 11b). The reason for the decrease in Δ CO₂ after the initial BB stimulation was that BB was turned on each time before the CO₂ concentration had declined to a base level. A longer off-interval would have eliminated this artifact, but this would also have defeated the purpose of repeated BB IR stimulation

Figures 11a, b. CO₂ output of lice during periodic stimulation under (a) aluminum and (b) blackbody radiators. Time periods were 2.5 min. Response delay represents the inherent lag on the system response due to air flow from the lice chamber to the CO₂ analyzer, it was approximately 1.1 min.

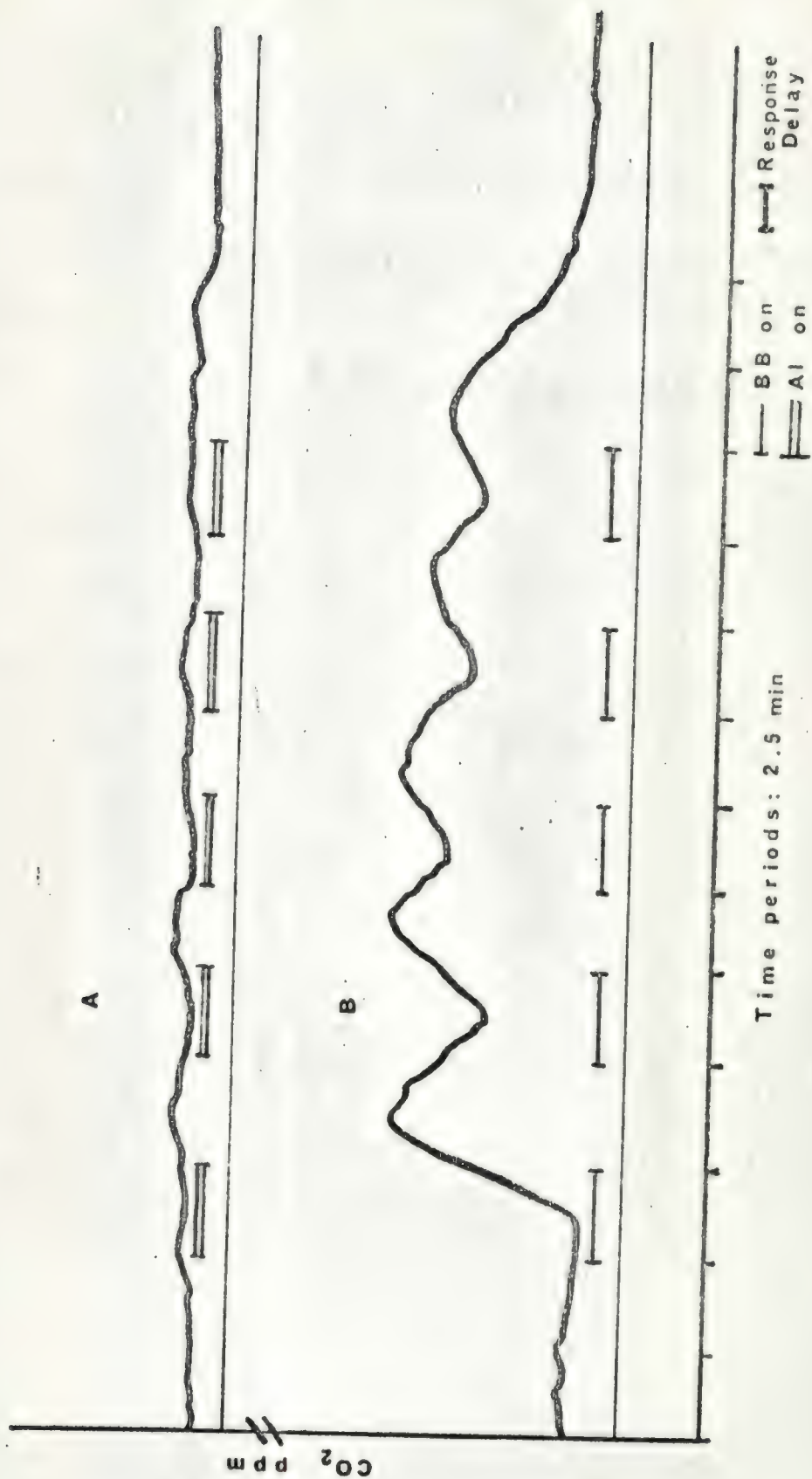


Table 7

Results of BB and A1 heaters stimulation upon 50, one-day-starved body lice.
 Values represent averages of readings taken every 2.5 min for 65
 min while either heater was on.

Heater	Temperature at time-off (C).			Change in Temperature			CO ₂ level at time-off (ppm)			Change in CO ₂ level		
	x	Sx	"t"	x	Sx	"t"	x	Sx	"t"	x	Sx	"t"
BB	26.23	0.17		0.63	0.10		62.0	6.88		9.5	3.38	
			0.27 N.S.			0.64 N.S.			10.73**			9.49**
A1	26.21	0.16		0.66	0.08		40.0	0.90		0	0.95	

**Highly significant at a 0.01 level.
 N.S. Not significant at a 0.10 level.

within short periods of time. Even so, the ΔCO_2 values were large enough to show the high degree of difference in the lice activity between BB and Al heating.

Four experiments were conducted, each with at least 10 replications, in which the BB and Al heaters were alternately turned on and off, with an off period in between them (see Figure 10c). All time periods were 2.5 minutes. Data from these experiments could not be pooled because of the great difference among their CO_2 baselines (33.0, 42.0, 32.5, 21.8 ppm). So, each experiment was analyzed separately using the recorded values of the temperature in the lice chamber when the heaters were turned on (T-on) and off (T-off). The change in chamber temperature was determined as the difference between T-off and T-on. CO_2 concentrations were recorded from the strip chart when the heaters were turned on (CO_2 -on) and off (CO_2 -off). The change in CO_2 concentration (ΔCO_2) was computed as a difference in concentration between heater off and on.

The value for T-off during BB-on and Al-on were significantly different (Table 8). The Δt values were also the same for the BB-on and Al-on periods. However, the CO_2 -off concentrations were greater during the BB-on periods. In three of the tests, CO_2 -off values were highly significant (at a 0.01 level) and significant in the fourth test

Table 8

Results on CO₂ output of lice when either the Al or BB heaters were turned on and off, alternately, with an off period in between.
All periods were of 2.5 min.

Parameter	1	2	3	4
CO ₂ baseline, S.				
T-off: \bar{x} , S. (BB)	33.0, 0.8	42.0, 1.7	32.5, 1.6	21.8, 1.3
\bar{x} , S. (Al)	26.19, 0.21	26.82, 0.13	26.33, 0.03	27.94, 0.10
"t".	26.18, 0.27	26.96, 0.10	26.25, 0.14	27.85, 0.20
Δt : \bar{x} , S. (BB)	0.05, N.S.	1.9, N.S.	1.14, N.S.	0.88, N.S.
\bar{x} , S. (Al)	0.71, 0.11	0.38, 0.08	0.77, 0.17	0.42, 0.04
"t".	0.80, 0.17	0.41, 0.10	0.62, 0.14	0.36, 0.11
CO ₂ -off: \bar{x} , S. (BB)	0.93, N.S.	0.50, N.S.	1.28, N.S.	1.15, N.S.
\bar{x} , S. (Al)	54.8, 5.5	48.8, 2.5	42.0, 6.2	43.2, 5.2
"t".	39.2, 3.9	44.0, 2.2	31.0, 1.1	32.0, 4.0
ΔCO_2 : \bar{x} , S. (BB)	5.15, **	3.21, *	3.51, **	3.41, **
\bar{x} , S. (Al)	18.0, 2.4	8.2, 3.6	11.5, 6.7	13.5, 11.7
"t".	-3.8, 3.6	3.0, 2.4	-0.3, 2.5	-5.6, 7.4
	9.1, **	2.7, *	2.9, *	2.8, *

N.S. Not significant at a 0.05 level.

*Significant at a 0.05 level.

**High significant at a 0.01 level.

(0.05). Subsequently, the CO_2 values were also greater during BB-on periods; but the ΔCO_2 values for the Al-on periods resulted in negative values in the majority of cases. These negative values were obtained because the lice did not react during the Al-on periods (see Figure 10c). This same effect was discussed in the previous experiment.

It can be stated that the environmental temperature did not have any effect on the kinetic state of the lice, which was related to CO_2 output, but it was the differential output of IR by the BB that made the difference.

The effect of human skin emanations upon lice behavior is summarized in Table 9. The values are the results of 10 readings with each of the two human volunteers, M. S. and K. F. The skin area, circumscribed by the chamber through which air was passed, was 9.61 cm^2 . Average output by these skin areas of the two volunteers were 9.2 and 8.4 ppm (average for the two, 8.8 ppm) when an air flow of 50 cc min^{-1} was maintained, representing $5 \times 10^{-5} \text{ cc of CO}_2 \text{ per minute}$. The human skin produces many volatile chemicals (U. S. Department of the Army, 1966); among them is lactic acid, which has been demonstrated to be an attractant for mosquitoes (Acree et al., 1968). When the lice were stimulated with human skin emanations under the experimental

Table 9

Effect of human skin air emanations on CO₂ output of 50 one-day-starved body lice (refer to Figures 14 and 15 for descriptions).

		CO ₂ (ppm)			
		Volunteer MS		Volunteer KS	
<u>Constants (a)</u>		<u>\bar{x}</u>	<u>Sx</u>	<u>\bar{x}</u>	<u>Sx</u>
Arm	(B)	9.2	0.45	8.4	0.89
Lice	(C)	18.5	0.88	33.4	0.99
Arm + Lice	(D)	29.5	1.37	41.5	1.20
<u>Experiment</u>					
Lice (during E)	(b)	19.0	1.30	33.4	0.55
Arm + Lice (D-off)	(c)	28.4	1.14	41.0	1.58
D-off-Lice (during E)	(d)	9.2	.84	7.6	1.67

- (a) Constants are values obtained by connecting directly the arm (B) or lice (C) to CO₂ analyzer. Arm + lice refers to the CO₂ concentration at D position during an experiment.
- (b) Values of CO₂ from lice during arm flushing prior to any experiment.
- (c) CO₂ values at end of time D (D-off) when the arm line was disconnected from the lice line.
- (d) Values of subtracting lice values from D-off (and obtaining similar value to arm B).

conditions, they did not become active, i.e., their CO_2 output did not increase above levels at an akinetic state (Table 9). This was shown by subtracting the skin and the lice CO_2 baselines from the CO_2 readings when the two, arm and lice, were connected to the line.

Reactions to an artificial finger
and varied stimuli

Lice reactions to stimulation by an artificial finger at 33 C were difficult to quantify. The majority of experiments were recorded as observations of behavior patterns and each type of observation was recorded from no less than 20 lice. Behavioral observations were recorded from normal lice and in some cases from lice whose last antennal segment, had been removed, and are referred to as antennectomized lice. It appears best to summarize and comment on these observations.

a. If a warm "finger" was slowly lowered to within 1.0 cm from an arena floor where lice were in an akinetic state, they began "awakening" over a circle with a radius of about 3 cm. If a cool "finger" was used instead, the lice remained in the akinetic state.

b. Normal lice followed a warm "finger" if it was maintained a short distance ahead in front of them. They did not follow or respond to a cool "finger." Similarly,

antennectomized lice followed a warm "finger" but not a cool one. If the lice began following a warm "finger" which was quickly replaced by a cool one, the lice continued following it momentarily. The cool "finger," however, would rapidly lose its attraction for the lice and they would move in another direction. If the procedure was repeated with antennectionized lice they kept following the cool "finger" for a longer period of time before losing interest and moving in another direction.

c. Normal lice followed a warm "finger" even in a situation when the arena was made of fine nylon and clean air was blown from underneath at a rate of $344 \text{ ml cm}^{-2} \text{ min}^{-1}$.

d. If normal lice were stimulated with a warm "finger" held 1.0 cm above the arena floor, the lice raised their heads and front legs, displaying an upward questing response (trying to grab). If this was repeated with antennectomized lice, they remained under the "finger," but they did not show the upward questing response.

e. If normal lice were crawling on a more or less straight course and encountered a spot that had been warmed by close contact with the warm "finger," they veered to either side but then returned again to the more-or-less straight line. This same behavior was shown by antennec-

tomized lice. This would be indicative of a klino-kinetic behavior.

f. When a warm "finger" was positioned on the vertical 0.5 cm from the arena floor and to the side of the general direction that normal lice were following, the lice either turned toward the "finger" or maintained the original straight direction. This depended on the angle subtended by the initial starting point of the straight line direction of the lice and the "finger." This means the angle measured was based on the following three points: (1) any point ahead on the line of movement, (2) the initial point of the lice movement, and (3) the position of the "finger" in relation to the line of lice movement. Of 30 measurements, 13 angles were greater than 37° and the lice maintained a straight course. The remaining 17 angles were smaller than 31° and the lice turned toward the "finger" and reached it. This experiment was repeated with antennectomized lice and the turning occurred only when the "finger" was positioned on the general direction of the lice movement.

g. If the "finger" was brought close from underneath to normal lice crawling on an arena floor made of nylon, the lice moved in circles around the "finger" position. When the "finger" was removed the lice reached and crawled

along close to the arena wall. The same behavior was observed with antennectomized lice. This suggests that perception of "warmth" can take place from underneath the lice.

h. When one-day-starved, normal lice were released on the skin of a volunteer's arm, they began feeding within an average time of 5.8 seconds (standard deviation, 2.3 sec). Initiation of biting was considered to be the time at which the lice arched their abdomen upward pushing against the substrate with the caudal tip of their abdomen. One-day-starved antennectomized lice (amputation performed the previous day) began feeding within an average time of 15.0 sec (standard deviation, 8.4 sec). A "t" test showed these time differences to be highly significant (0.01 level).

i. Neither normal or antennectomized lice fed on an arm skin made cold with ice.

j. When a heater tape (at 33 C) was covered with the upper portion of a rubber glove, which is very smooth, lice did not attempt any feeding. But if the heater was covered with the inner palm area of the glove, which is very rough, the lice initiated feeding behavior. Both normal and antennectomized lice showed this behavior.

The significance of blackbody IR detection

An established fact is that when a hand is brought

close to akinetic lice they become, within seconds, very active. Behavioral experiments, reported in this study, demonstrated that lice became very active when stimulated by blackbody IR radiation. Experiments also demonstrated that lice attempted feeding on warm, clean rubber, but did not show any feeding behavior when the human skin was cooled with ice. All these facts raise doubts over the value of olfaction in host detection and in feeding stimulation. The facts also point to the importance of blackbody IR as a token stimulus in host detection by body lice.

Howlett (1917) pointed out the advantage of lice getting excited when stimulated by "warmth," the main advantage being "in securing the wider distribution of the species by infecting anyone whose body came in contact with that of their host." Of course, the advantage is not to the species, but to the louse which wanders away from its host.

Air currents carrying lice eggs on falling hair or cloth threads is a common mode of dispersion. The newly hatched larvae have to find their host. Hence, the reaction to blackbody IR is advantageous, because by actively moving only when the host is in close proximity, it increases the chances of rewarding the energy expenditure with a blood meal and economizing energies in the absence of a host. If the size and means of locomotion of lice are considered from

their host-detecting capacity, it is seen that it would not be advantageous for the lice to be always on the move, i.e., in an excited and continuously moving state. The lice detected blackbody IR at least 3.25 cm away but not a 4.75 cm. As previously quoted, several workers, experimenting with non-flying blood-sucking insects, found that these insects usually detected the hosts "warmth" between 4-5 cm away. There are three possible explanations for this threshold distance. (1) It would not be advantageous for the lice to detect and respond to the host from a far distance, because the lice could not cover in an efficient period of time the distance separating them from the host. The lice could detect the host only when it was close. The probability of rewarding the energy expenditure by being excited would then be higher. This would require a distance discriminator built in the lice sensory system. The mode of action of this distance discriminator could be based on energy level, as energy decreases inversely to the square of the distance. (2) A second possible explanation could be if the lice sensory system were limited on the energy level it could detect. (3) A third possibility could be a limitation on the energy level of the blackbody IR as the result of some unaccounted properties of blackbody IR and its interaction with the environment, i.e., air, moisture, host.

Another problem in host detection encountered by lice is finding the host's skin when the lice are located in the outer garments. Body lice live on the surface of a man's body or clothing, although often they are found on their host's bedding (Buxton, 1946). Nuttall (1917) stated that eggs (nits) are found generally attached to the fabric of the outer garments and that the larvae have to find the skin to feed. Lice living in this garment milieu are constantly exposed to the emanations from the host's skin. Odor stimulus under these conditions may not offer information toward directionality of its origin. Blackbody IR from the skin provides for a directional stimulus for the lice that have to thread their way through garments to reach the skin. When an infested person is dying or has died, or if the person has fever, the lice abandon him (Buxton, 1946; Nuttall, 1918). The "heat sense," the wärmesinn of Frickhinger, is distributed over the body, that is, it is not restricted to a given part of the louse body. Distribution of this sense over the body allows for the detection of warm surfaces touching the louse body. The antenna appears to contain a good portion of the "heat sense." Previous works (Wigglesworth, 1941; Homp, 1938) indicated loss of temperature discrimination after complete antennectomy and the present research showed behavioral changes after the removal of the fifth antennal segment.

The antenna morphology

The first antennal segment of the body louse has the two common antennal muscles, the erector and flexor. They are anchored inside the head and distally to the joint between the first and second segments (see Figure 13). Three small setae forming a triangle are located near the proximal end of the segment. These are the 1-7 group in Figure 12. These 3 setae are difficult to locate because they are frequently covered by the antennal socket. On the mesodorsum of the segment there is 1 tactile seta (1-6 of Figure 12) and more distally, near the articulating membrane, 5 more setae. Some of these last setae are angulated forward (setae 1-1 and 1-3, Figure 12). Each one of these tactile setae, as well as the others in the rest of the antenna, are innervated by one sensory cell (Plate 5). A wide and scalloped articulating membrane is present between the first and second segment. This scalloped membrane does not entirely cover the distal portion of the first segment, since there are two small portions on the lateral sides in which the cuticle extends almost contiguous with the next segment (Figure 12, Plate 2).

The second segment bears a total of 10 setae. One of them, the 2-10 of Figure 12, does not occur until the 3rd instar.

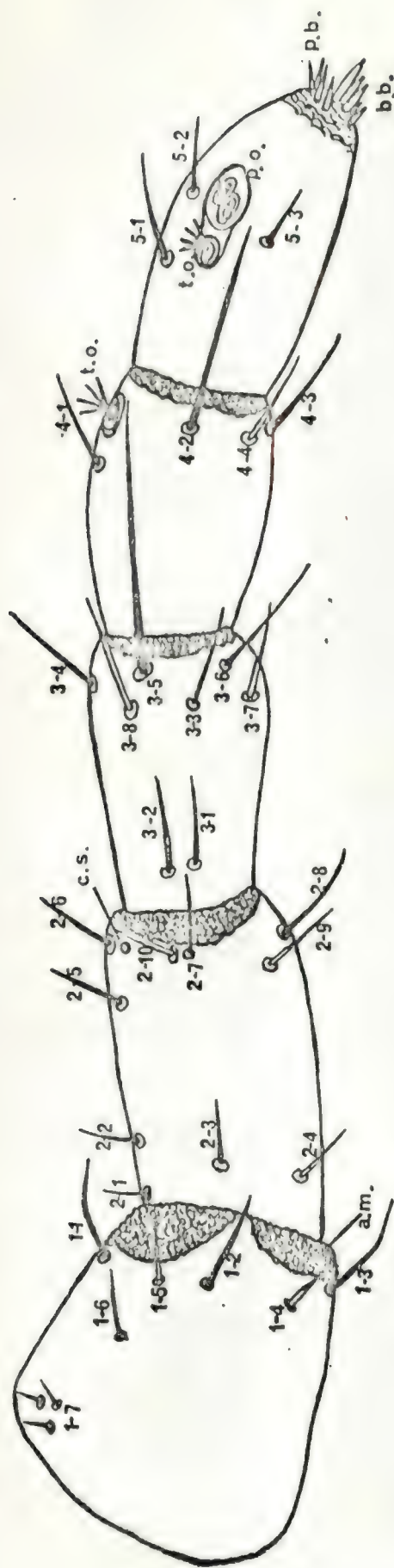


Figure 12. Diagram of adult body lice antenna. The setal numeration is arbitrary within a segment. am, articulating membrane; bs, blunt setae; cs, campaniform sensilla; po, pore organs; ps, pointed setae; to, tuft organs.

Plate 1. Dorsolateral view of left antenna of adult body louse. Scalloped area between segments is articulating membrane. SEM X 230




Plate 2. Ventrolateral view of left antenna of adult body louse. Arrow points to the area where the articulating membrane is lacking, i.e., the pivot for the movement of the second segment. SEM X 250






Plate 3. Fourth and fifth segments of adult body louse antenna. Circular area on the distal portion of the fourth segment is depression where tuft organ is centered. Similar, but not circular, depression areas are found around tuft and pore organs on the fifth. Pointed and blunt setae are seen on the tip of the fifth segment. SEM X 600

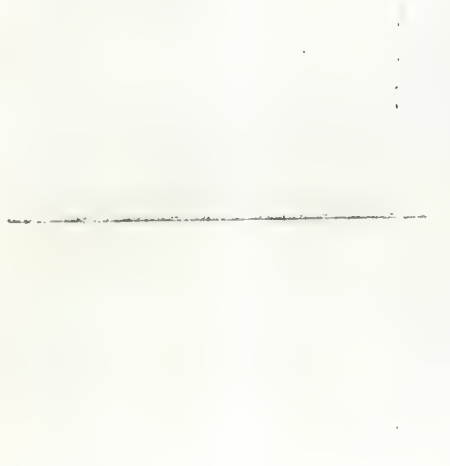
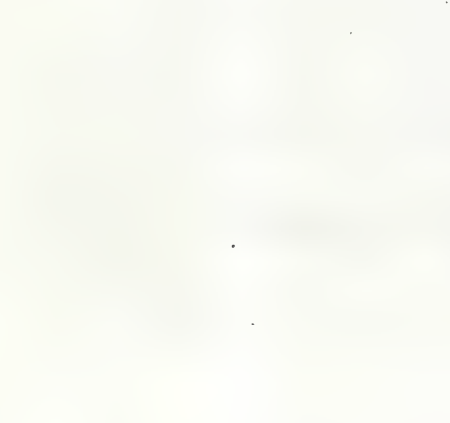


Plate 4. End view of antenna tip of 1st instar larva of the body louse. Two blunt setae are lacking. All the pointed ones are present when the larvae hatch. SEM X 3,000

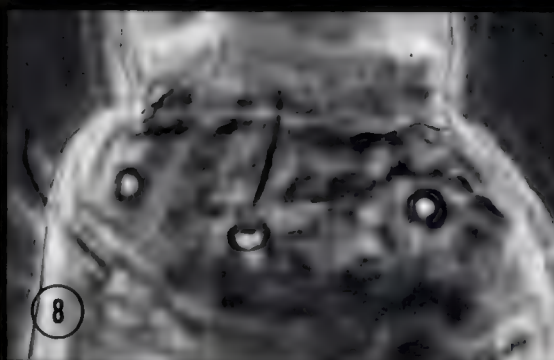
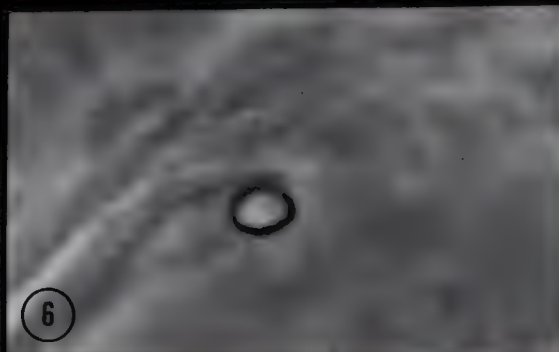
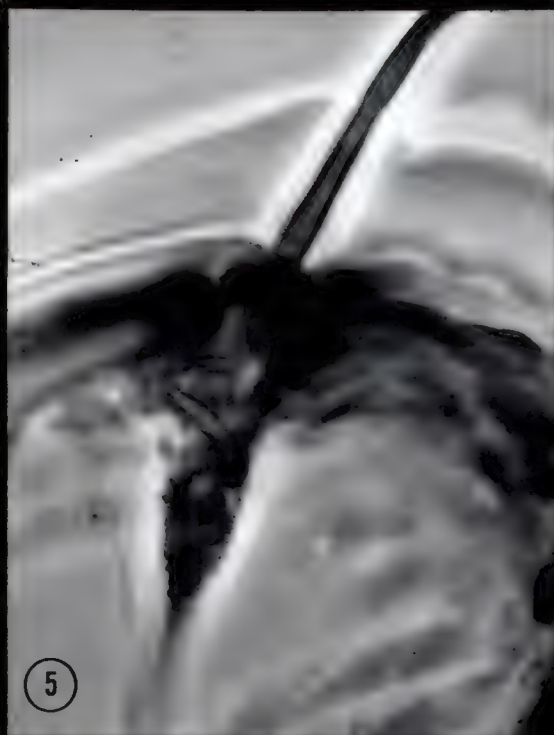




Several chordotonal organs are located in this segment. A double chordotonal is located close to the ventral wall of the segment. Two single chordotonal organs are located in each venter and dorsum of this segment. These organs are attached to supporting tissues that run proximally across the segment, giving an appearance of an internal wall (Plate 10, Figure 13). These organs are connected to the antennal nerve near these supporting tissues.

There are two structures on the distal part of this segment which have the appearance of campaniform sensillae. They were not described by either Keilin and Nutall (1930) or Wigglesworth (1941). I originally thought they were anchoring bodies of the chordotonal organs, but their location did not correspond to the distal portions of the chordotonal organs. These campaniform organs are seen under phase as two bright birefringent bodies, very similar to hair sockets (Plates 6 and 8). Under Nomarski interference differential contrast, they appear much like any tactile hair socket (Plate 9). Snodgrass (1935) stated that campaniform sensillae resemble in surface view vacant hair follicles. They are, however, extremely difficult to locate with the scanning electron microscope (SEM). The only possible way to find them is by searching the segment area lying tangentially to the observer (Plate 7). They cannot be

- Plate 5. Antennal tactile hair (h), innervating dendrite (d) and neuron cell (c); axon (a) extends toward antennal nerve. Epon-araldite section. Ropell-Carlisle stain procedure. Phase contrast. X 1,600
- Plate 6. Campaniform sensilla on the second segment of the antenna of the body louse. It shows strong birefringency under phase contrast. Non-fixed specimen. X 1,600
- Plate 7. Campaniform sensilla on the second segment of the antenna of body louse. It can be located with the SEM only on a tangential view, but not on normal incidence. SEM X 2,820
- Plate 8. Campaniform sensillae on the second antennal segment, demonstrating their high birefringency, similar to the hair sockets between them. Phase contrast, non-fixed specimen. X 640
- Plate 9. Same view as Plate 8, but under Nomarski differential interference contrast. It demonstrates the striking similarity to the hair socket between them. X 640
- Plate 10. Internal components of the second antennal segment: (d) double chordotonal organ; (s) single chordotonal organ anchored on supporting tissues (t) which form a pseudo-wall across the segment; (tr) trachea; (n) antennal nerve. OsO_4 treated whole antenna. Nomarski differential interference contrast. X 640
- Plate 11. Color photomicrograph of double chordotonal organ on the second antennal segment. OsO_4 treated whole antenna. Phase contrast. X 1,600
- Plate 12. Color photomicrograph of single chordotonal organ on the second antennal segment. OsO_4 treated whole antenna. Phase contrast. X 1,600



recognized with an SEM if studied under normal incidence to the observer. The SEM shows only the outermost cuticular layer, and the campaniform sensillae appear lying under the cuticle. They are located distally and dorsolaterally, close to the articulating membrane (Figure 12).

Pringle (1938) determined that campaniform sensillae are sensitive to tangential strains in the cuticle. Wigglesworth (1965) stated that when they are radially symmetrical (as is the case in the body lice) they react to flexion equally in all directions. The function of these organs located dorsolaterally in the second segment may be the reception of cuticle stresses during antennal movements, adding to the information supplied by other sensors, about the antenna position at any given moment.

Adult body lice appear to have a band of articulating membrane between adjacent antennal segments. In 1st instar larvae the last three segments of the antenna lack this articulating membrane and appear as if fused into one. This articulating membrane appears in the 2nd and following instars, but the width of this band between segments 3 and 4, and 4 and 5, is narrower than between 1 and 2, and 2 and 3. The third segment carries 8 tactile setae. One of these, 3-5 of Figure 12, is close to 100 μ m long and is located dorsally. Four of the setae on this segment, 3-1,

3-2, 3-3, and 3-8 of Figure 12, do not appear until the 3rd instar. This segment bears only tactile hairs, and no other known sense organ.

The fourth segment carries 5 tactile hairs. One of them, 4-2 of Figure 12, does not occur until the 3rd instar. A tuft organ is located distally on the dorsum of this segment (Plate 3). The fifth and last segment bears the greatest number and variety of sense organs. Three tactile setae, none longer than 40 μ m, are present from the 1st instar and are located dorsally (Figure 12, Plate 3). An extended depressed area appearing concave with SEM runs along the dorsum of the segment (Plate 3). A tuft organ is located proximally in this depression, and distally, two pore organs. Between the pore organs and the tuft organs there is a small ridge (Plates 3, 4). The distal portion of this segment is slightly bent downward. This section has a scaley appearance as seen under SEM (Plates 3, 23), which cannot be resolved with the light microscope. Ten basiconic setae are found on the apex of this segment. Six blunt setae are set more-or-less ventrally on the apex. These blunt setae arise from an area devoid of the scaley type of terrain. Only 4 of these blunt setae are present in the 1st instar larva (Plate 4). Dorsad to the blunt setal group, there are 4 pointed setae arranged in a

semicircle (Plates 3, 4). These are present in the 1st instar larvae.

The antennal nerve bifurcates about the second or third segment. Fine tracheae run all the way to the sense cells underlying the blunt and pointed setae on the tip of the antenna.

Species comparison study

A survey of body and head lice from different parts of the world was carried on to add information on the number of tuft organs on the antenna. Wigglesworth (1941) claimed that there were 4 tuft organs on the body louse antenna and Miller (1969) found only 2. This survey was also intended to find any difference between head and body lice antennae. The following is a list of specimens examined (borrowed from the U. S. National Museum, Washington, D. C., or obtained from other sources):

Pediculus humanus (female). On man (head). Calcutta, India, 1945.

P. humanus (male). On man (head). Calcutta, India, 1945.

P. humanus (both sexes). On man. Congo, 1965.

P.h. corporis (both sexes). On man. Washington, D. C., 1942.

P.h. humanus (both sexes). On P.O.W. Korea, 1951.

- P.h. humanus (both sexes). On vagrants. Japan, 1951.
- P.h. humanus (both sexes). On man. Puerto Rico, 1935.
- P.h. humanus (both sexes). On beggar. Peking, China, 1924.
- P.h. humanus (both sexes). On human. Luzon, Philippine Islands, 1953.
- P.h. humanus (both sexes). "Orlando Regular." 1951.
- P.h. capitis (both sexes). Durango, Mexico, 1918.

In all the specimens studied, the setal count was identical for blunt setae (6), pointed setae (4), tuft organs (1 on the 4th, 1 on the 5th) and pore organs (2 on the 5th). Head lice specimens which were collected from human heads in San Salvador, El Salvador, 1970 (Mauricio Salazar, coll.), and from a child's head in Gainesville, Florida, 1971 (A. Broce, coll.) were studied and displayed the same number and types of sensors. Specimens of body lice from the Freetown, Korean and Burundi colonies had the same sensor complement. Only one body louse of the standard colony of the more than 200 studied showed an abnormality in the sensor complement. This louse had two tuft organs on the fourth segment (Plate 20). Otherwise, its antennal structure was normal.

Although Wigglesworth (1941) did not state the source of the specimens used in his study, they probably were European in origin. Unfortunately, no specimens from this

part of the world could be obtained for study. However, the cosmopolitan distribution of human lice leaves little doubt that the discrepancy between his description and that of Miller's (1969), on the number of tuft organs on the antenna, was due to a matter of misinterpretation, that is, taking the pore organs for tuft organs. Not a single difference was found on the antennal sensor complement between body and head lice.

Function of the antennal proprioceptive organs

Wigglesworth (1941) suggested the possibility that the chordotonal organs in the second antennal segment probably served to make the insect aware of its own antennal movements. The following is an analysis of the antennal movements and how the chordotonal organs may accomplish the proprioceptive function. As explained earlier, the body louse antenna has muscles only in the first segment (the scape of more complex antennae). The erector and flexor muscles control the up-and-down movements of the second and subsequent segments as a unit. The cuticular areas of the distal border of the first segment extending to reach the second segment serve as pivotal points for the up-and-down movement of the distal four segments (Figures 12, 13, Plate 2). That is, the distal portion of the antenna moves

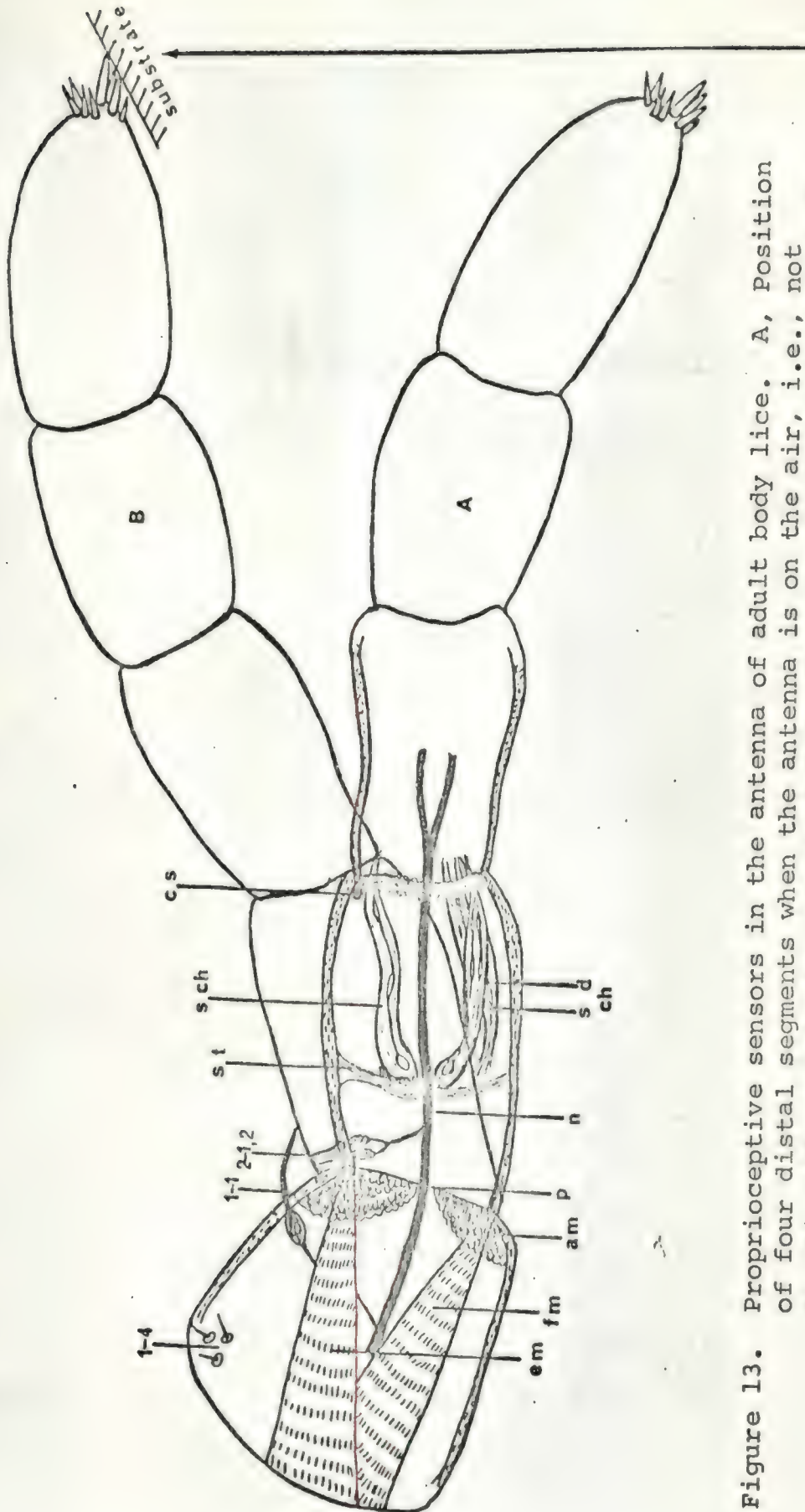


Figure 13. Proprioceptive sensors in the antenna of adult body lice. A, Position of four distal segments when the antenna is on the air, i.e., not touching the substrate. B, Position of the four distal segments when the antenna is touching the substrate. See text for explanations on the mode of action of the proprioceptive sensors. am, articulating membrane; cs, campaniform sensilla; em, erector muscle, fm, flexor muscle; n, antennal nerve; p, pivot (fulcrum; s-d ch, single and double chordotonal organs; 1-4, 1-1, 2-1, 2, tactile hairs; st, supporting tissues.

about a horizontal axis. These cuticular extensions are situated on the lateral sides of the segment. Individual movement of the distal four segments cannot be accomplished because they lack the musculature. The antenna can be moved as a whole (including the first segment) by a series of muscles attached to the head and the proximal end of the first antennal segment. This movement controlled by the muscles is mainly on a horizontal plane, i.e., about a vertical axis, although an up-and-down movement is possible. The antenna also moves, of course, whenever the head is turned from side-to-side and up-and-down.

The group of 3 small setae (1-7 of Figure 12, 13) set close to the antennal socket are constantly in touch with the articulating membrane. There are numerous setae on the head proper and around the antennal socket laying flat against the articulating membrane. These two groups of setae supply information about the position of the first segment relative to the head.

When the louse lowers its antenna, the first portion to touch the substrate are the blunt setae on the tip of the antenna. The last antennal segment is bend downward, as is shown in Plate 2. The blunt setae are set further ventrally on the extreme portion of this segment, as shown in Plates 2, 3, and 23. The antennal tip, highly close-up magnified

in Plate 23, is particularly interesting because it shows how the tip of the blunt setae lay on a more-or-less straight line. Plate 23 is an oblique view which gives the impression that the tip of one of the setae is not on the same plane as the others. The pointed setae do not touch the substrate under normal conditions. A study of the location and orientation of the tactile hairs indicates that when the antenna tip is in contact with the substrate, they (the tactile hairs) do not reach the substrate. As will be explained in a later section, the blunt and pointed setae do not possess the characteristic structures of mechanoreceptive sensillae. Hence, the information about when the antenna is touching the substrate has to come from another part of the antenna. Here the chordotonal organs come into play.

Once the antenna has touched the substrate and the flexor muscle keeps exerting pressure, the most likely joints to bend are the ones between the first and second segment, and between the second and third. Information about the upward bending of the second segment, in relation to the first, could be supplied by the small seta on the second segment and laying dorsad against the articulating membrane (seta 2-1 of Figures 12 and 13). A stronger curvature would cause some of the bent setae on the first

segment, specially seta 1-1 of Figure 12 and 13, to touch the second segment. There is considerable bending freedom for the antenna at the joint between the second and third segments; at least, greater than at the two other distal joints. The joints between the third and fourth and the fourth and fifth have a narrower band of articulating membrane. The bending of the joint between the second and third segments exerts a stretching force on the double and single chordotonal organs, located on the ventral part of the second segment. This stretching action is translated into nerve impulses and transmitted to the antennal nerve.

Body lice live generally between skin and clothing or between layers of clothing. It is necessary for the louse to know the position of its antenna relative to the stratum above it. This information could be obtained from some of the proprioceptive setae on the first segment, and the single chordotonal organ in the second segment. This organ could convey the information if the antenna is bent in an upward movement. The fact that the chordotonal organ is single in the dorsal portion of segment two and complex in the ventral portion leads to several possible explanations. It is more important for the louse to know when the antenna is touching the floor than when it is touching the "ceiling," since the louse would need more information about the

substrate, i.e., the food source. When a louse is crawling on a surface at a normal gait, it lowers its antennae and touches the substrate at very short intervals. High speed cinematography revealed that the louse did not interrupt its gait when it touched the substrate with its antennae. Therefore, the antennae are bent backwards after touching the substrate and this information may be important for the louse. This information could also be provided by the double chordotonal organ, since its distal end bifurcates toward the lateral sides of the segment. There are several long setae on the dorsum of the antenna, especially on the last three segments. These setae could provide some information about the position of the antenna when touching the "ceiling." As stated earlier, no ventral tactile setae could touch the substrate and thus it appears as if the blunt setae do not have mechanoreceptive functions (inferred from ultra structure studies).

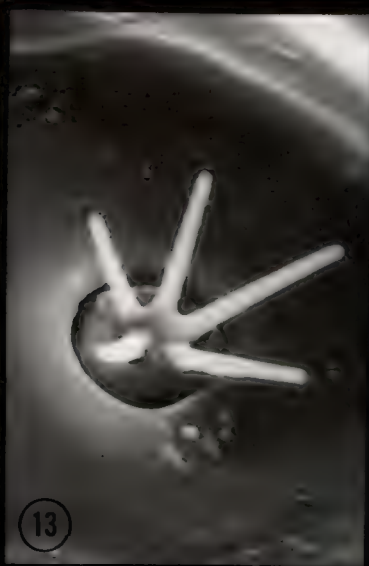
The tuft and pore organs

Each tuft and pore organ has 4 cells associated with it. These 4 cells are inside a sac-like structure and so closely associated that they appear to lack cell walls between them. There is no visible difference between the cell bodies associated with the tuft and with the pore organs. Each organ appears to be innervated by a double-

stranded chord, that is, 2 dendrites (Plate 16). The tuft arises from a cone and protrudes through a rounded hole on the cuticle (Plates 13, 16, 17). No difference was found between the tuft organs of body and head lice. Abnormal tufts are found occasionally. The abnormality consists of some of the hairs being fused. Plate 14 depicts the fusion of 3 hairs of a tuft from a head louse. This type of abnormality was also reported by Roth and Willis (1951) in the tuft organs of Tribolium spp. A pore organ consists of a rounded hole which extends downward as a tube (Plate 15). Underneath the hole, there are some spaces around the tube. In cross section, they appear much like a tuft organ (Plates 18, 21). This similarity may have been the reason that Wigglesworth described 4 tufts, but no pore organs. When the louse molts, the fine hairs of the tuft and the tube of the pore organs are shed and readily visible in the exuvia (Plate 19).

Miller (1969) found heavy precipitations around the openings of the pore organs under SEM. I found precipitation around these organs only on those specimens prepared for gold-coating (Plate 15). I never observed it in unprepared specimens; it appears to be due to the fixation procedure. An inherent problem of the SEM is that many unexpected artifacts are produced.

- Plate 13. Tuft organ on fifth segment of antenna of adult body louse. Gold-palladium coated. SEM X 6,000
- Plate 14. Abnormal tuft organ on fifth antennal segment. Gold-palladium coated. SEM X 12,400
- Plate 15. Pore organ on fifth segment of antenna. Note "secretion" from pore, due to fixation. Gold-palladium coated. SEM X 23,000
- Plate 16. Cross section of tuft organ on the fifth segment. Observe dendrite (d) entering tuft shaft. Epon-araldite embedded. Ropell-Carlisle staining procedure. X 1,600
- Plate 17. Cross section of tuft on the fifth segment. Mallory triple stain. X 1,600
- Plate 18. Cross section through pore organ on the fifth segment. OsO_4 fixation. X 1,600
- Plate 19. Antenna of 3rd instar larva of body louse undergoing molting, where the old cuticle (exuvia, e), the new cuticle (n.c.), canal of pore organ on exuvia (p), and exuvia of tuft (t) are seen. X 640
- Plate 20. Abnormal louse with 2 tufts found on the fourth segment. Tuft on the fifth segment is visible on the left of the photograph. Nomarski differential interference contrast. X 1,000



The peg sensors

The 10 peg sensillae on the tip of the antenna have two distinct types of tips. A group of 6 setae possess blunt nipple-like tips (Plate 46). These 6 setae are set on the extreme tip of the antennae. They are located ventrad with their tips pointing downward toward the substrate (Plates 3, 23). The diameter of their base ranges from 1.9 to 2.5 μm and their length from 6.3 to 15 μm (Figure 14). These 6 setae are located on an area devoid of the scaley terrain characteristic of the tip of the antennae. As explained earlier, the tip of these blunt setae lay on a straight plane which corresponds with the substrate when the antennae are lowered. Therefore, seta b-1 of Figure 14 is set dorsad to the rest of the blunt sensillae and is the longest. Two setae in this group, b-5 and b-6 of Figure 14, are small and their tips are not as conspicuously blunt as the rest. The 1st instar larva possesses only 4 of these blunt setae. The b-5 and b-6 are missing (Plate 4) and they appear after the first molt. The dimensions of the blunt setae on the 1st instar larvae are the same as in the adult stage.

The remaining 4 peg setae possess pointed tips. They are located dorsal to the blunt group and surrounded by scaley terrain (Plate 23). They are more uniform in size

Figure 14. Diagram illustrating the arrangement of the blunt (b) and pointed (p) setae on the tip of the antenna of adult body lice. Positions of the basal spots are indicated by arrows. Setal dimensions represent average of a minimum of 5 and a maximum of 25 measurements per seta.

Setae	dia.	length	μm
b1	2.5	15.	
b2	2.5	12.	
b3	2.5	10.	
b4	2.2	9.5	
b5	1.9	7.	
b6	1.9	6.3	
p1	2.3	8.8	
p2	1.9	8.8	
p3	2.2	8.8	
p4	1.9	8.2	

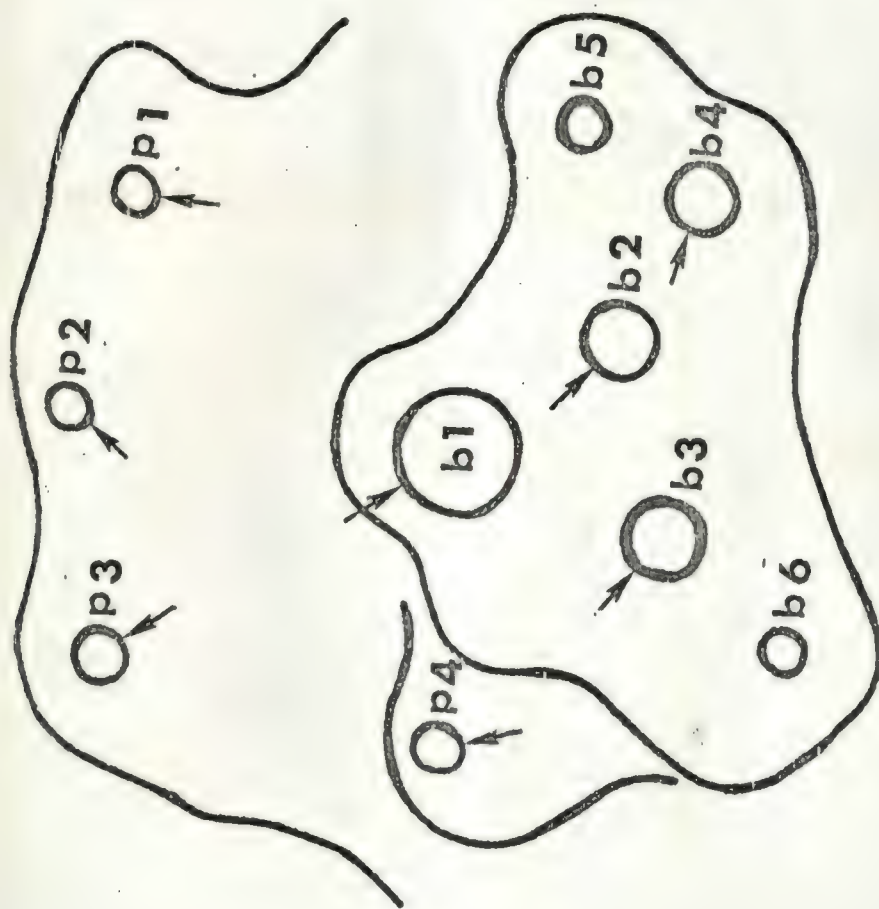


Plate 21. Distal three segments of the antenna of 1st instar larva. They appear fused into one segment. n: antennal nerve; t: trachea. Nomarski interference differential contrast. X 400

Plate 22. Tip of the fifth segment of body lice antenna showing how the peg sensillae give the impression of thin-walled sensors. Basal spot is visible on the center sensilla. Group of nerve cells under the pegs are also visible. Non-fixed specimen. Phase contrast. X 1,600

Plate 23. Tip of antenna of body lice. Observe the characteristic scaly terrain. Black line indicates the relative position of the substrate when the antenna is lowered and the blunt setae touch it. SEM X 2,470

Plate 24. Longitudinal thick section of fifth segment of body lice antenna showing the cell bodies underlying the peg organs. The basal spot can be seen in two of the pegs. Epon-araldite embedded, Ropell-Carlisle stain procedure. Phase contrast. X 640

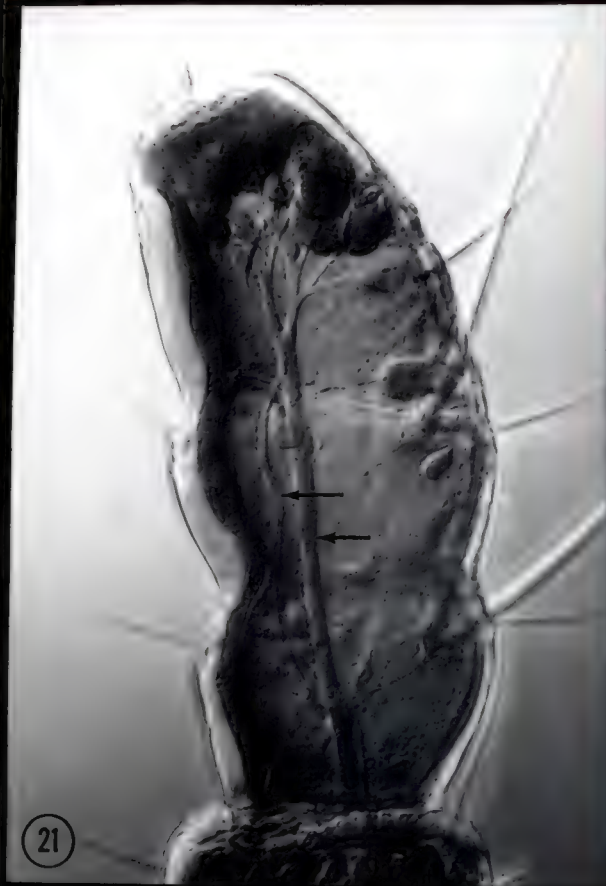
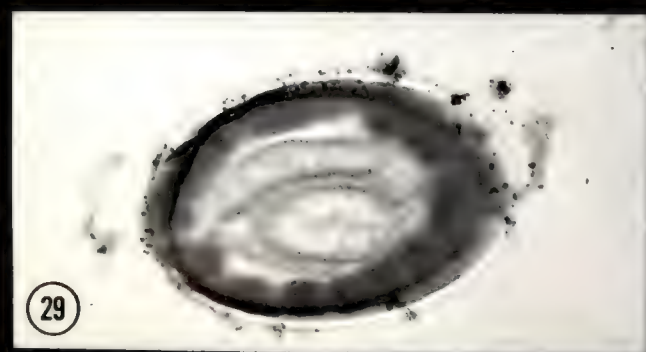
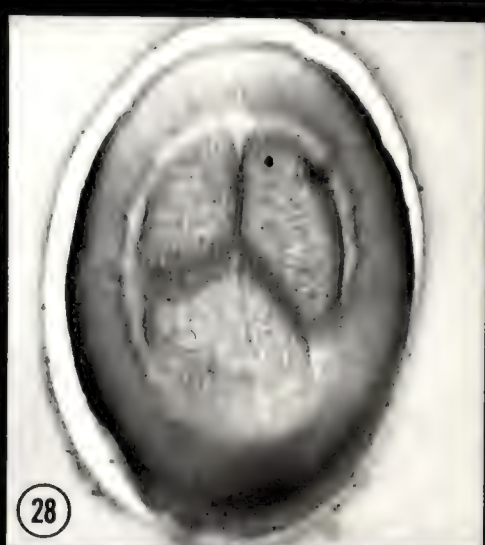
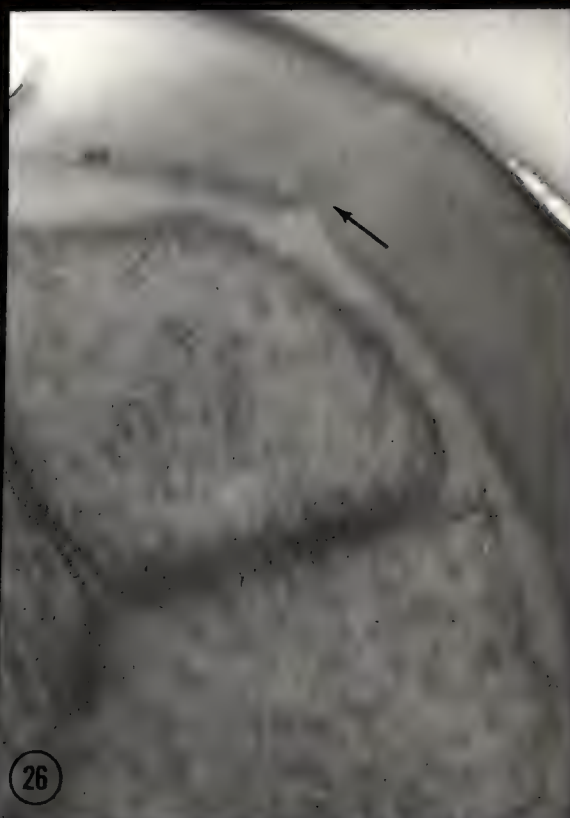


Plate 25. Highly magnified pointed seta from adult lice antenna. Observe the hole-perforated appearance of the sensor cuticle. OsO_4 treated specimen. SEM X 11,900

Plate 26. Cross section of a pointed seta from adult body lice. Arrow points to a narrow pore through the cuticle. Note the microtubules within the 3 dendrites and the lack of a cuticular sheath enveloping these dendrites. TEM X 78,500

Plate 27. Oblique section of a pointed seta through basal spot area. The 3 visible dendrites are above the basal spot. TEM X 25,000

Plates 28, 29 and 30. Cross sections of pointed setae of adult body lice. Numerous pores are visible on their thick cuticle. The 3 dendrites are the most common number in this type of setae, although 2 dendrites are not uncommon. TEM Plate 28, X 28,000; Plate 29, X 24,000; Plate 30, X 20,000



and appearance than the blunt ones. Their base diameters range from 1.9 to 2.3 μm and they are 8.2 to 8.8 μm long. One of these setae, p-4 of Figure 14, is set laterad of the blunt group. The remaining 3 pointed setae are located on a horizontal plane and dorsad to the blunt group. The 1st instar larva possesses the 4 pointed setae.

The peg setae have near their bases a "spot" characteristic of the thin-walled (basiconic) sensillae. This is the basal spot through which the cuticular sheath is evaginated during molting. The position of the basal spot is constant for any given seta. The spot in the pointed seta is somewhat ventrad, but on a blunt one, dorsolaterad (arrows in Figure 14). The basal spots of the smaller blunt setae, b-5 and b-6 of Figure 14, were difficult to locate under SEM. Although difficult, the other spots could be located under light microscopy (Plate 22, 24), but were easier to find under SEM (Plates 23, 46).

Results of the tests for permeable areas in the peg sensillae were negative. The crystal violet technique of Slifer (1960), the aniline blue of Callahan's (P. S. Callahan, personal communication, 1970) and the silver nitrate method of many authors gave negative results. The pressure technique of Slifer, Prestage and Beams (1957) for open tip thick-walled sensillae also failed to show any

permeable area. Washing the specimens with detergent, prior to treatment with the dyes, also failed to show any permeable area on the tip or any other area of the sensor. It was puzzling that not even the basal spot could be observed to stain with the different dyes, even when the specimens were soaked in the dyes for several hours. This type of test for permeable areas is currently being used to determine if a sensor has an olfactory function. Foelix (1970), in finding that some spider's sensillae stained heavily with crystal violet through their tips, considered that such an open tip was a strong argument for a chemoreceptive function.

Each peg sensor was found to be innervated by a group of cells. But the exact number of underlying cells could not be determined (either 5 or 6) (Plates 22, 24). No difference could be observed under light microscopy between the cells innervating the pointed setae and the ones innervating the blunt ones. Studies of the peg sensillae with the transmission electron microscope (TEM) were most frustrating, because of poor fixation. Even when fixation time was doubled, the preparation was poor. The major problem was the dimensions of the antenna. This problem is not common with other types of insect antennae, because the antenna width is greater and penetration of the fixatives

is facilitated. Because the body louse antenna is so small, penetration took place only through the cut end. Penetration of the fixatives into the pegs was further complicated by their diameter. Another serious problem encountered was that after osmication (OsO_4) the pegs became very brittle and fell off. Much material was wasted by cutting with the glass knife. However, when a diamond knife was used the thick sensors could be sectioned for the TEM.

Studies of the pointed setae with the TEM indicated that these setae have a lumen occupied by 2 or 3 dendrites (Plates 26, 27, 28, 29, 30). They possess a thick wall (0.31 to .37 μm) which is perforated by few narrow pores (Plates 26, 28, 29). These pores widen when reaching the internal walls of the sensillum. They are inconspicuous under SEM observation (Plate 25).

The dendrites occupying the lumen of these sensors in cross section are full of microtubules. No cuticular sheath was found covering the dendrites, at least in the portion distal of the basal spot. No microtubules were found leaving the dendrite bundle toward the walls.

TEM studies of the cells underlying the peg setae indicated that they possess the characteristic components of sensory cells. Three and four dendrites were found innervating each one of these sensors (Plates 35, 36, 40). The

usual 9 doublet structure was found in several of the specimens. Plate 31 shows a cross section of 3 neuron cells, through the ciliary region; 2 of the dendrites have attained the doublet structure, while the other still has the basal body structure. This indicates that the dendrites attain the different structures at different levels. Plate 32 shows 3 dendrites all with the 9 doublet structure. They are seen covered by the cuticular sheath, as a thick electron-dense membrane. These doublets separate and form the single microtubules seen in Plates 33 and 34. These last two plates show how the cuticular sheath envelops each one of the dendrites. The dendrite in the upper part of Plate 33 possesses about 38 microtubules. Therefore, these microtubules have separated and multiplied once. It is interesting to note the peculiar arrangement of the microtubules in this dendrite. No explanation can be offered for the low number of microtubules in the center dendrite. The lack of microtubules in the remaining dendrite is due to poor fixation. Slifer and Sekhon (1969), who have published extensively on the subject of ultrastructure of insect sensors, commented on this problem, that "Sections of dendrites sufficiently well fixed and so oriented that every fibril can be counted with certainty are obtained only rarely."

Plate 31. Ultra-thin section of a group of 3 neuron cells. This section is through the ciliary region. Two dendrites have the couplet structure and the third one has the basal body arrangement. TEM X 31,700

Plate 32. Section through ciliary region of nerve cells innervating the peg sensilla. The 3 dendrites have the doublet structure. Observe the heavy cuticular sheath covering the outer edges of the dendrites. TEM X 65,000

Plate 33. Section of dendrites bundle beyond the ciliary region. Note the heavy cuticular sheath covering individually each dendrite and the unusual arrangement of microtubules on the dendrite at the top of the plate. About 38 microtubules may be counted on this dendrite. TEM X 31,700

Plate 34. Section of a 2-dendrite bundle of a peg sensor cells. Letters indicate the succeeding steps in microtubule multiplication by splitting (see text for explanation). Observe heavy cuticular sheath enveloping each dendrite individually. TEM X 65,000

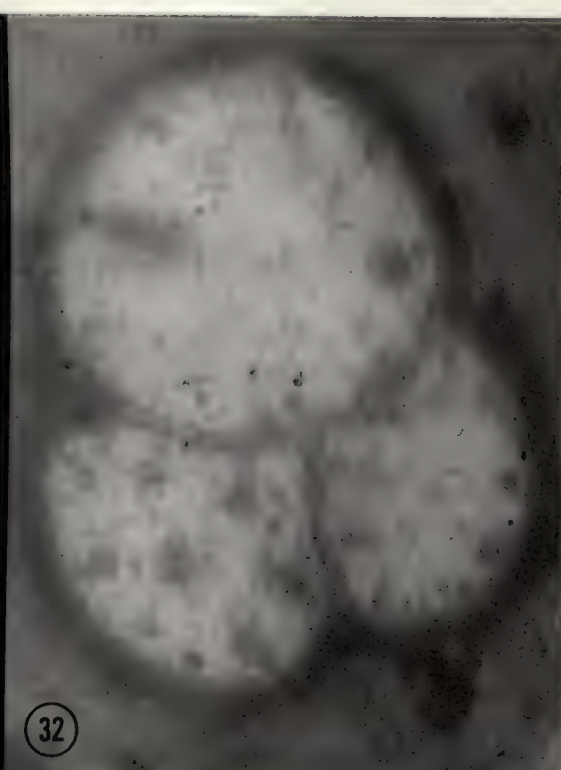
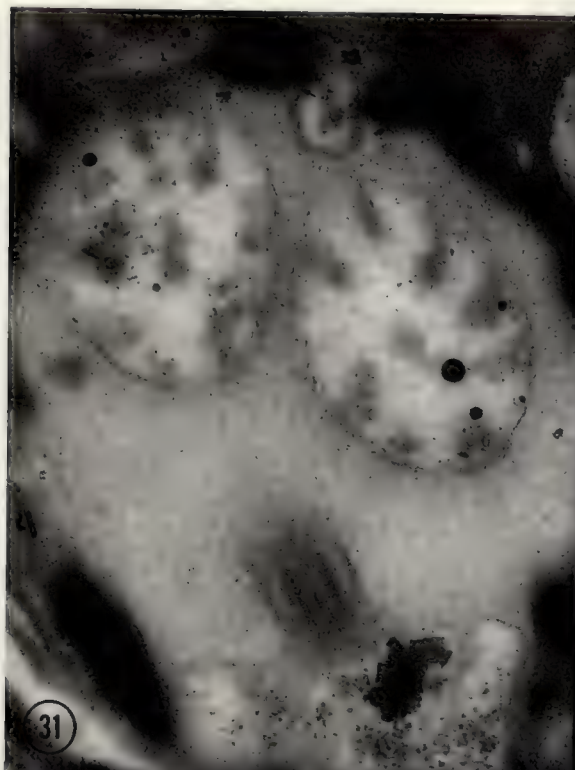


Plate 35. Cross section through the tip of antenna of body louse. Several dendrite bundles are seen. These bundles are formed by 2, 3, or 4 dendrites. The 2-dendrite bundle on the lower right part of the plate innervates a pointed seta. Large open spaces on the center are vacuoles found around the nerve cells of the blunt setae. TEM X 4,000

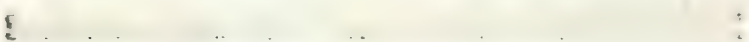
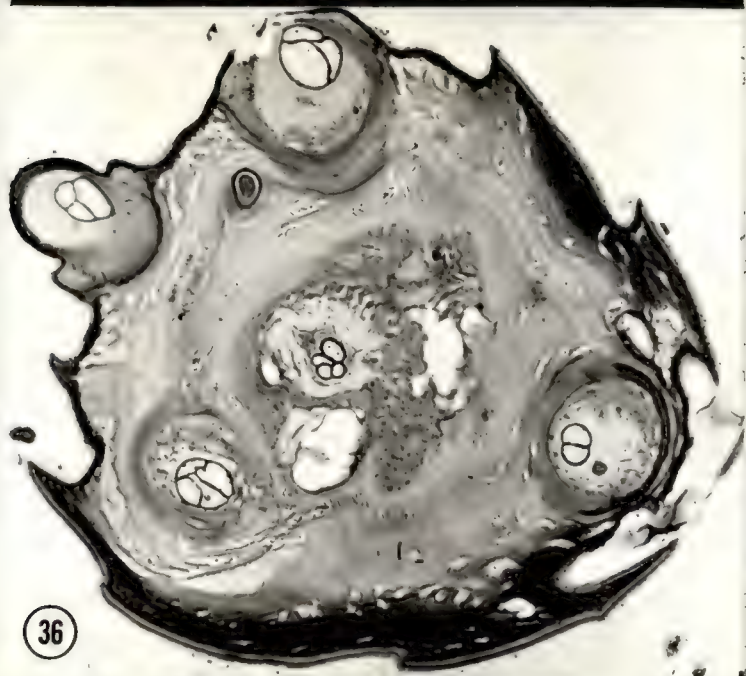
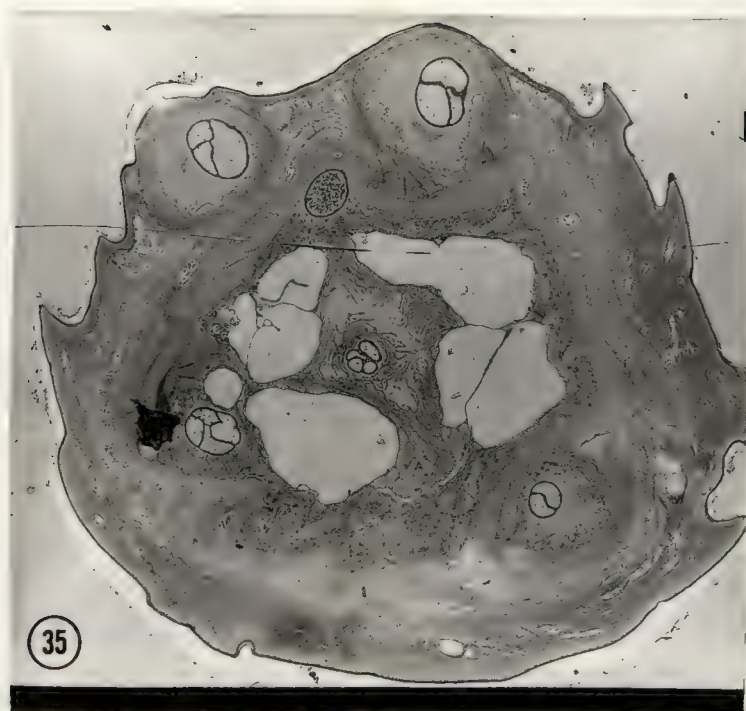


Plate 36. Same as cross section in Plate 35, but distally to it. Base of some of the blunt setae may be seen. TEM X 4,000



The mechanism of microtubule multiplication has been difficult to interpret. Slifer and Sekhon (1969) considered two possibilities: "(1) additional fibrils may appear de novo and be in no way associated with the original 18 fibrils: or (2) each fibril may branch to form two and these, in turn, may branch again." These authors believed the second possibility to be more plausible. Plate 34 adds evidence to their beliefs that the microtubules multiply and are a continuation of the cilium. Arrow (a) points to a normal, single microtubule. Arrows (b) point to microtubules with a wall across them, while arrows (c) point to microtubules that have increased in size with more conspicuous septa. Finally, arrows (d) point to further development in the microtubule splitting, just before completing the separation. This Plate 34 is presented as evidence that microtubule multiplication takes place by fibril splitting, growing and finally, separation.

Cross sections of the tip of the antennae showed that some vacuoles are present surrounding some of the nerve cells underlying the blunt sensors, especially the largest peg seta, b-1 of Figure 14 (Plates 35, 36). The cuticular sheaths covering the dendrites are seen in Plates 35 and 36. Plate 39 shows the cuticular sheath in more detail. In certain preparations, the cuticular sheath appeared double

layered, with connections between the two layers (Plate 39). The blunt setae appeared under SEM as if they were articulated, that is, as if they had a flexible membrane at the base (Plates 22, 48). The lack of an articulating membrane was demonstrated by TEM (Plates 36, 37, 38).

Cross sections of the blunt setae were more difficult to obtain than the pointed ones. They appeared to have thick walls (about 0.35 μm). No pores were found on their walls, ruling out the possibility of their being thin-walled (basiconic) sensillae, as previously reported. Three or four dendrites were observed entering the lumen of these sensors (Plates 37, 38, 40-45). Plates 41 and 44 are of the same sensor, at different levels, but very close to the basal spot, particularly Plate 44. On this picture, only 1 dendrite can be seen, the others were lost during sectioning. If Plates 41 and 45 are compared, it is seen how the dendrites fill the lumen of the sensilla in Plate 45, but not in Plate 41. It appears that the dendrites become narrower when they approach the basal spot. The dendrites have to pass through the cuticular sheath in order to continue toward the tip of the sensilla. After passing through the cuticular sheath, the dendrites widen again. If this is the way dendrites travel along the sensilla, a peculiar problem arises. The microtubules multiply before reaching

Plate 37. Longitudinal and somewhat oblique section through the base of a blunt seta. No articulating membrane is observed at the base. Four dendrites are seen entering the peg lumen. TEM X 20,000

Plate 38. Longitudinal section of a peg sensor. No articulating membrane visible at the base. TEM X 8,000

Plate 39. Section through bundle of 2 dendrites prior to entering a pointed seta. Observe double-layered cuticular sheath and microtubules inside the dendrites. TEM X 51,000



Plate 40. Cross section through peg sensors. Seta on lower left corner is a pointed seta, while the rest are blunt ones. TEM X 4,500

Plates 41 and 42. Cross section of blunt setae in the proximity and proximal to the basal spot. Narrowing of the dendrites is readily visible. The cuticular sheath is also seen covering the dendrites. Observe also the thick walls devoid of pores. TEM Plate 41 X 31,500; Plate 42, X 41,000



Plate 43. Cross section of same sensor as in Plate 41, but through the basal spot (on the left). The position of this basal spot corresponded to its position observed with the SEM. Only 1 dendrite remains, the other 3 have been lost during sectioning.
TEM X 30,000

Plate 44. Cross section of same sensor as in Plate 42, but nearer to the basal spot. Observe 1 dendrite with just 1 microtubule. TEM X 51,250

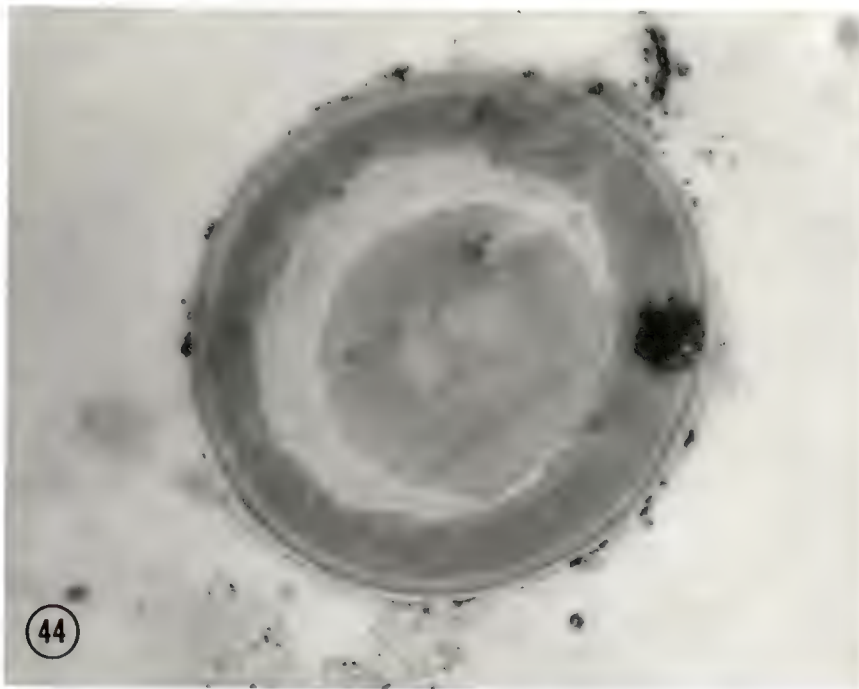
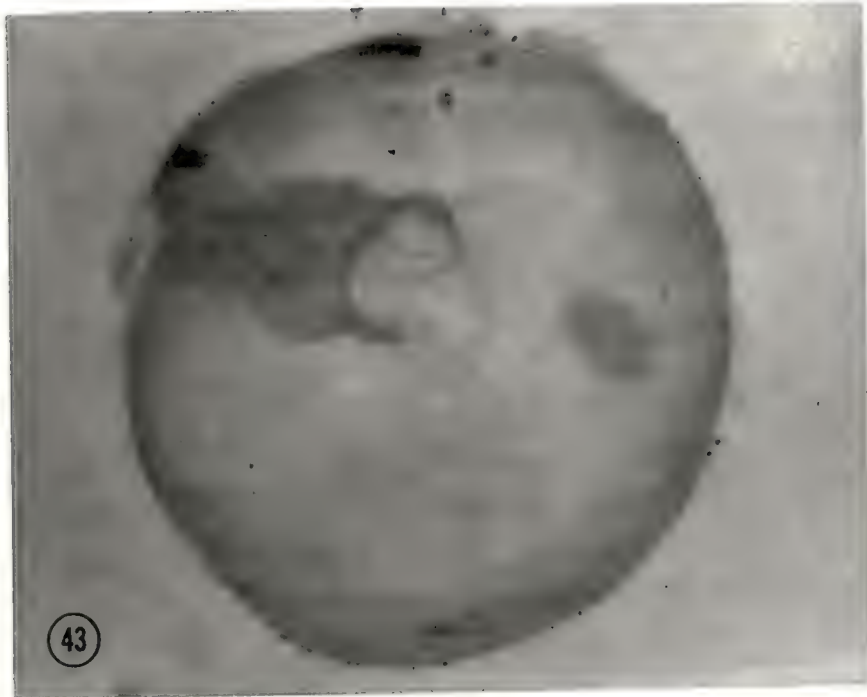


Plate 45. Cross section of blunt sensor, distad to the basal spot. Observe the great difference in microtubule numbers in each dendrite. No cuticular sheath is present at this level. Observe thickness of the sensor walls. TEM X 42,000

Plate 46. SEM view of nipple-like structure at the tip of the blunt setae. The basal spot is also observed. SEM X 14,000

Plate 47. Longitudinal section of the nipple-like tip of a blunt seta. Dendrites are not resolved but sensor inner walls are readily seen closing under the tip. TEM X 28,000



the basal spot area, but once they reach this area they reduce in number. Once they cross the cuticular sheath they would multiply again. This should not seem unrealistic if we accept Slifer and Sekhon's theory that the function of these microtubules is to provide support to the dendrites (1969). Sonication of antennae fixed with osmium tetroxide (OsO_4) eliminated some of the blunt setae. One of the specimens observed in the SEM is relevant to this section. Plate 50 shows the dendrites of a blunt setae which broke off. Two dendrites can be made out of the structure arising from the sensor socket. A constriction appears at the end of these dendrites.

The nipple-like structure at the tip of the blunt setae appeared very bumpy under the SEM (Plate 46). Considerable difficulty was encountered in sectioning this nipple-like structure. Only one section was obtained for study with the TEM. This was a longitudinal section and somewhat oblique in nature and is shown on Plate 47. It is a poor preparation in which the dendrites are not clearly visible. But the inner walls of the sensor are recognizable and seem to be closed under the nipple structure. The poor fixation in this area adds supporting evidence to the fact that these sensors are impermeable at the tip. Although the evidence is not conclusive, it does indicate that the

Plates 48, 49 and 50. Different aspects of OsO_4 treated and sonicated antennae of body lice. Plate 48 depicts a fallen peg and some dendrites arising from the socket. Plate 49 shows a detached peg with a shrunk appearance. Plate 50 is interesting as it shows dendrites arising from the socket. They seem to constrict which would correspond to the basal spot constriction. SEM Plate 48, X 6,000; Plate 49, X 11,000; Plate 50, X 12,000

Plate 51. Antenna of 3rd instar larva molting into adult. New antennal cuticle is seen on the left of the Plate. The evaginated (molted) cuticular sheaths appear as streaks running from the new sensors to the base of the old pegs. Observe that the new pegs are larger than the old cuticle. Phase contrast. X 640



dendrites are not exposed at the tip of the blunt sensillae, as has been shown in other insects such as Phormia regina (Dethier, 1955) and in spiders (Foelix, 1970).

Sonication of osmicated (OsO_4) specimens dislodged many sensors. Plate 48 shows a blunt seta halfway separated from the antenna. At least 2 dendrites are seen arising from the socket. They appear as if they had shrunk probably due to the high vacuum the specimens are subjected to in the SEM. Naked dendrites can also be seen on Plate 50. To my knowledge this is the first time that dendrites have been observed under the SEM. Some sensors were completely dislodged from their positions, but remained on the antennal surface. Some of these had an appearance of being squeezed or shrunk (Plate 49).

Some specimens were obtained that were in the process of molting. Plate 51 shows an antenna in the process of molting. All the complement of sensors is present in the new cuticle. One noticeable difference was that the blunt setae were longer than the old cuticle, by a factor of up to 25 percent. The new cuticle must shrink, since it was found that the setae in the different instars had the same dimensions as those of the adult.

No conventional name was applied to the pointed and blunt setae on the fifth segment. This was avoided purposely

for two major reasons. First, the subject of sensor nomenclature is in such a state of confusion that further terminology would add to the confusion. Secondly, these sensors possess peculiar characteristics which could classify them in different categories of sensillae. The basal spot is a strong character of the thin-walled sensilla. But the thick-wall of the blunt setae (devoid of pores) classifies them as sensilla trichodea. The pointed setae, with their wall perforated by few pores, would be classified as trichodea, but their basal spot is a characteristic of the thin-walled (basiconic) sensillae. The lack of permeability of the tip of these sensors prevents them from being classified as thick-walled sensillae (according to Slifer).

Wigglesworth (1941) considered that these peg sensors were olfactory receptors. It is difficult to accept this fact after the negative results obtained with the dye penetration techniques. A highly disturbing fact in this research was finding with the TEM that the pointed setae possessed few small pores, but these sensillae did not show any permeable area under the dye penetration tests. The fact that the dye penetration tests were performed so many times, under different conditions and using different dyes, strengthens the case against dye tests being infallible in determining pores.

CHAPTER V

CONCLUSIONS

This research contradicts previous research in demonstrating that body lice do detect infrared radiation of a blackbody nature. They orient toward the blackbody IR radiation source. Detection was demonstrated by monitoring the CO₂ output as an index of locomotive activity. Good correlation was obtained between CO₂ output and blackbody IR stimulation. IR detection was also demonstrated by the lice orientation to the blackbody IR source. Detection and orientation toward the IR source was accomplished in the absence of convective heat. Detection and orientation to the IR source took place at distances greater than 3.25 cm but smaller than 4.75 cm. This distance agrees with the threshold distance reported for other blood-sucking insects, such as bedbugs (Rivnay, 1932) or mites (W. Bruce, in preparation, 1971).

IR radiation acts as a stimulus for the lice, indicating that a warm-blooded food source is close by, and providing a means of orienting to it. It brings the lice out

of the akinetic state into a very active state of searching, increasing the chances of their reaching the IR source, i.e., the food source. Convective heat is not necessary for the host's "warmth" detection. IR detection is not exclusively localized at the distal segment of the antenna, but may be distributed all over the body, including the first four antennal segments. Elimination of the fifth antenna segment modified the lice behavior. Whether this was the result of the loss of sensors (at least four different types) on this segment or the effect produced by the amputation trauma is not known. At least, amputation of the fifth antennal segment showed that sensors on this segment may be involved on IR detection for directional movement toward the IR source. Since one of the main functions of antennae is to provide directional "orientation" information (Callahan, 1967), it is probable that they are involved in the reception of the blackbody IR radiation. Wigglesworth (1941) concluded that the pegs sensillae on the fifth segment were odor receptors. However, antennectomized lice fed as well as normal lice on the skin, the only difference being the time required to start feeding. Either these pegs are not odor receptors or lice do not need the added stimulus of smell to elicit a feeding response.

Food source (skin) temperature is important to elicit

feeding behavior on lice as was demonstrated by the cooling experiments. Wigglesworth (1941) showed the lice preference for rougher materials. Feeding response, at least probing, is affected by substrate roughness as lice probed on warm, rough surfaces but not on warm, smooth surfaces.

Body lice, Pediculus humanus humanus, L., and head lice, P.h. capitis, possess the same number of peg, tuft and pore organs on the antenna: One tuft organ each on the fourth and fifth segments, 2 pore organs on the fifth, 4 pointed and 6 blunt peg setae on the fifth segment. Lice from different laboratory colonies and from different parts of the world have the same sensory complement.

The only ontogenetic difference found was in the number of blunt setae on the antennae of the 1st instar larvae. These larvae possess only 4 blunt setae. The remaining 2 setae appear after the first molt. The dimensions of the setae remain constant throughout the different instars and adult stage.

The lice antennae possess several chordotonal organs, campaniform sensillae and tactile hairs that act as proprioceptors and provide the lice with information about the position of the antennae at any given moment.

The peg sensillae on the fifth segment have no permeable areas demonstrable with any reported dye or stain

techniques, raising questions about their reported role as chemoreceptors. The pointed setae possess few pores on their thick walls and are extremely narrow. This disproves the infallibility of the dye tests. The blunt setae do not even have these narrow pores on their thick walls. A basal spot is found on the pointed and blunt setae. This is the site of the cuticular sheath evagination during molting. The cuticular sheath covers the dendrites up to the basal spot region, and, from there on, the dendrites are in close contact with the sensor walls. These peg sensillae are innervated by 2, 3, or 4 dendrites. The dendrites do not branch inside the pegs.

The number of microtubules inside the dendrites increases to more than 100 per dendrite in some, but just above 18 in others. Evidence was presented that demonstrated that microtubule multiplication takes place by microtubule splitting.

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BIOGRAPHICAL SKETCH

Alberto Bolivar Broce was born on January 2, 1942, in Las Tablas, Republic of Panama. He graduated from the Instituto Nacional de Panama in 1959, and upon graduation he was awarded a scholarship by the Escuela Agricola Panamericana in Tegucigalpa, Honduras, C. A., and graduated from that institution in December, 1962, with the degree of Agronomo.

He attended for seven months a training program on tropical research at the Tropical Research Department of the United Fruit Company in Honduras.

With the aid of a scholarship granted by the Escuela Agricola Panamericana he entered the University of Florida in the fall of 1963, and completed the requirements for the Bachelor of Science degree in August, 1965.

In the fall of 1965, the United States Department of Agriculture, through the Florida Experimental Station, granted him a research assistantship to continue studies toward the Master of Science degree.

He was an assistant during the I.A.E.A./F.A.O. International Short Course on radiation and radioisotopes in Entomology in 1967 and 1969. He participated as a lecturer to a similar short course held in Turrialba, Costa Rica, in 1970.

Married to the former Bobara Anne Mergenthal, he is the father of two sons. He is a member of the Entomological Society of America, the Florida Entomological Society, Phi Sigma, and Alpha Zeta.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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